Extracts of Terminalia arjuna and uses thereof

Field of Invention

The invention relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing diseases such as cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart diseases, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; owing to the extracts antioxidation potential.

The invention also relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing various infectious diseases.

More particularly, the invention relates to certain extracts from *Terminalia arjuna*, their uses as antimicrobial agents and antioxidants for the treatment of certain diseases like cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract in mammals, particularly humans, processes for obtaining them and delivery formats therefore.

Background

Antioxidant potential

Reactive oxygen species (ROS) are a family of molecules including molecular oxygen and its derivatives produced in all aerobic cells. Excessive production of ROS, outstripping endogenous antioxidant defense mechanisms, has been implicated in processes in which they oxidize biological macromolecules, such as

DNA, protein, carbohydrates, and lipids. This condition has commonly been referred to as oxidant stress. An increasing body of evidence suggests that oxidant stress is involved in the pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, hypertension, diabetes, and heart failure.

Many ROS possess unpaired electrons and thus are free radicals. These include molecules such as superoxide anion ($O_2^{\cdot-}$), hydroxyl racial ($HO^{\cdot}$), nitric oxide ($NO^{\cdot}$), and lipid radicals. Other reactive oxygen species, such as hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and hypochlorous acid (HOCl), are not free radicals per se but have oxidizing effects that contribute to oxidant stress. The cellular production of one ROS may lead to the production of several others via radical chain reactions. For example, reactions between radicals and polyunsaturated fatty acids within cell membrane may result in a fatty acid peroxyl radical ($R\text{-}COO^{\cdot}$) that can attack adjacent fatty acid side chains and initiate production of other lipid radicals. Lipid radicals produced in this chain reaction accumulate in the cell membrane and may have a myriad of effects on cellular function, including leakage of the plasmolemma and dysfunction of membrane-bound receptors. Of note, end products of lipid peroxidation, including unsaturated aldehydes and other metabolites, have cytotoxic and mutagenic properties.

In mammalian cells, potential enzymatic sources of ROS include the mitochondrial respiration, arachidonic acid pathway enzymes lipoxygenase and cyclooxygenase, cytochrome p450s, xanthine oxidase, NADH/NADPH oxidases, NO synthase, peroxidases, and other hemoproteins. In addition to endogenous oxidative stress, exposure to free radicals and oxidants in the environment, such as ultraviolet sunlight, ozone, cigarette smoke, smog, and other pollutants, also contribute substantially to the rate of change in the body's oxidant: antioxidant balance. A shift in the oxidant: antioxidant balance due to increased production of free radicals may contribute to the decline of cardiovascular, neuronal, muscular, visual, and immune functions, over time. In addition, a high level of oxidative

stress and free radicals has been implicated in an ever-widening array of age-related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract. (Am J Clin Nutr 2000; 71 (supll): 1665S-8S.)

Detoxification of ROS by antioxidants therefore affords protection against such diseases. There is a growing body of evidence suggesting that antioxidants contribute to cardioprotection.

Atherosclerosis, a chronic inflammatory disease of the arterial wall, is a major cause of morbidity and mortality from cardiovascular disease (CVD) in much of the world's population. Atherosclerosis is a complex process that leads to heart attack, stroke, and limb loss by the plugging of the arteries with atherosclerotic plaque. There have been several reports indicating oxidation of Low Density Lipoprotein (LDL) as one of the major mechanisms responsible for the pathogenesis of atherogenesis. The hypothesis that oxidative stress plays a role in atherosclerosis rests on the inference based on experimental work, on a large scale, carried out in animal models of heart disease and by extension, antioxidants by their ability to quench free radicals and reactive oxygen species, may play a beneficial role in modulating oxidative damage and thereby decreasing the risk of atherosclerotic lesion formation and progression. (J. Nutr. 131: 366S-368S, 2001.)

Nitric oxide (NO) is produced from L-arginine in the vascular endothelium by the endothelial iso-form of nitric-oxide synthase (NOS). Endothelial production of NO is crucial in the control of vascular tone, arterial pressure, smooth muscle cell proliferation and platelet adhesion to the endothelial surface. Impaired endothelium-derived NO bioactivity is a common feature of many vascular diseases that is thought to contribute to their clinical manifestations, as evidenced in a study conducted to investigate the effect of ascorbic acid on NO synthesis. The study also revealed that ascorbic acid was shown to enhance impaired

endothelium-dependent vasodilatation in patients with atherosclerosis by a mechanism that is thought to involve protection of NO from inactivation by free oxygen radicals. Ascorbate pretreatment on endothelial cells led to a 3-fold increase of the cellular production of NO measured as the formation of its co-product citrulline and as the accumulation of its effector molecule cGMP. It was thus shown that intracellular ascorbic acid enhances NO synthesis in endothelial cells and that this may explain, in part, the beneficial vascular effects of ascorbic acid. (J. Biol. Chem. Vol.274, No.12, Issue of March 19, pp. 8254-8260, 1999., J. Biol. Chem. Vol.275, No. 23, Issue of June 9, pp.17399-17406, 2000.)

Degenerative neurological diseases affect millions of people around the world. A number of these diseases, including amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), Parkinson's disease, and Alzheimer's disease, appear to have ROS toxicity as a central component of their underlying mechanism of nerve cell destruction. Unfortunately, there is little evidence that simply eating more dietary or even pharmocologic antioxidants will prevent or arrest the neural degeneration; not surprisingly the mechanism is too complex to lend itself to such a simplistic remedy. Nevertheless, improving our understanding of these complex injury mechanisms offers a real potential for improved clinical outcomes in the near future.

Ischemia/reperfusion injury is a particularly fascinating example of ROS-mediated disease. When an organ is deprived of its blood supply (ischemia) it is injured, not just by the temporary loss of oxygen, but also by the ROS that are generated by reaction with the oxygen that is reintroduced at reperfusion, when the blood supply is restored. In some clinical situations, we can prevent this injury by giving antioxidants, sometimes even *after* the period of ischemia, but just prior to reperfusion. For example, the preservation of kidneys, livers, and other organs in solutions that contain antioxidants, as well as other agents, is now routine prior to their transplantation. Another example is the use of drugs that block the function of free radical generating enzymes prior to stopping the heart for cardiac

surgery. These drugs help prevent reperfusion injury when the heart is restarted and flow is restored. This reperfusion injury mechanism also has been found to play an important role in patients suffering from multiple organ failure after trauma, massive surgery, or shock. Multiple organ failure is now the leading cause of death in intensive care units, and extensive efforts are under way to understand better how ROS contribute to this syndrome.

Aging is a process *per se*, i.e., a series of controlled mechanisms, and not just the passive accumulation of wear and tear over the years. Put simply, our bodies age in the ways that are far more complex and more regulated than the processes by which our automobiles wear out. But if aging is a series of processes, it's logical to conclude that it is potentially controllable, or at least modifiable. One of the most important of these processes is comprised of an accumulation of the molecular injuries that are mediated by free radicals and other ROS. For example, since structural lipids are the primary component of our cell membranes, the integrity of which defines cell viability, aging is partially a matter of our going rancid as our lipids are progressively oxidized. While this is an oversimplification of this complex process, it reflects the optimism of some investigators of the aging process.

Recent studies indicate that the therapeutic manipulation of ROS metabolism can actually extend the total life span of mice to a significant degree. This was the first time that life span has been successfully altered experimentally by treatment. When one considers that the demographic, and consequent social, economic, and ecological impacts of even a 10 percent increase in human life span, a likely eventuality within the next decade or two, would far exceed that of a 100 percent cure for cancer (which is far less likely), the importance of this potential becomes evident.

As the understanding has evolved, it would provide unprecedented opportunities for improving the quality and even the length of human life.

Antibacterial Potential

Resistance to existing drugs is developing at an alarming rate. Thus, a diverse arsenal of new antibacterial agents is urgently needed to combat the diminishing efficacy of existing antibiotics.

In India, herbal medicines have been the basis of treatment and cure for various diseases/physiological conditions in traditional methods. Although reports of antibacterial activity of indigenous plants have been published from many regions, they have not been systematically conducted, except in a few cases.

Terminalia arjuna plant extracts

Terminalia arjuna is a deciduous tree found throughout India growing to a height of around 60-90 feet. *Terminalia arjuna* belongs to the family *Combretaceae* and is called "Arjuna" in vernacular. *Terminalia arjuna* has been used for over 1500 years in India as a cardio tonic and has been indicated for derangement of all three humoursin, vata, pitta and kapha in Ayurveda. The bark of *Terminalia arjuna* has been widely used in Indian system of medicine for a variety of purposes.

Sharma VN et al. evaluated the antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree bark (a popular cardiotonic substance in Indian pharmacopoeia) and compared it with a known antioxidant, vitamin E by a randomised controlled trial. It was concluded from this trial that, *Terminalia arjuna* tree bark powder has significant antioxidant action that is comparable to vitamin E. In addition, it also has a significant hypocholesterolaemic effect. (Antioxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: a randomised placebo-controlled trial, J Assoc Physicians India 2001 Feb; 49:231-235)

The bark of Terminalia arjuna tree has a long history of use as a cardiac tonic as well, and has been indicated in the treatment of coronary artery disease, heart failure, hypercholesterolemia and for relief of anginal pain. (Miller, A. L. Botanical Influences on cardiovascular disease. Alternative Medicine Review. Dec 1998, vol 3. No. 6, pages 421-431.

Ethanolic extract of Terminalia arjuna tree bark in doses of 100 mg/kg and 500 mg/kg significantly reduced total and LDL cholesterol levels in hypercholesterolaemic rabbits. (Ram et al. Hypocholesterolaemic effects of Terminalia arjuna tree bark. Journal of Ethnopharmacology. Vol 55. No. 3, pages 165-169.)

It is reported that the bark of T. *arjuna* exhibited antibacterial activity only in dichloromethane, methanol, and aqueous extracts against *E.coli, K. aerogenes, P. vulgaris, P. aerogenes* at 1000-5000 ppm dosage. But there is no reference to the antibacterial activity of Ethyl acetate extract and other solvent extracts than mentioned above. Also there are no reports of the effect of *Terminalia arjuna* bark extracts on Gram positive bacteria. Additionally there are no reports of the effect of *Terminalia arjuna* fruit extracts on gram positive or gram negative bacteria (Samy et. al. Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethanopharmacology 62 (1998) 173-182.).

It is reported that the bark of *T. arjuna* exhibited antioxidant activity only in direct aqueous extract as determined *invitro* by DPPH radical scavenging and deoxyribose damage protection assay and *invivo* by effect on lipid peroxidation. In the present invention direct and successive extracts except direct aqueous extract of *T. arjuna* bark and fruit have shown potent antioxidation activity (Munasinghe et. al., Antiradical and Antilipoperoxidative Effects of Some Plant Extracts used by Sri Lankan Traditional Medicinal Practitioners for Cardioprotection. Phytotherapy Research 15 (2001) 519 –523) .

There exists a need for the development of new medicines, which are effective in treating diseases like cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract.

Summary of the invention

Objects of the invention will become apparent from the following description and examples.

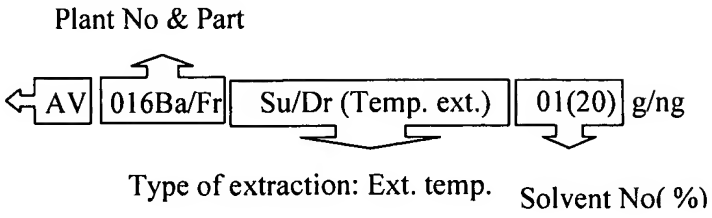
The invention relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing diseases such as heart disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; owing to the extracts antioxidation potential.

The invention also relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing various infectious diseases.

More particularly, the invention relates to certain extracts from *Terminalia arjuna*, their uses as antimicrobial agents and antioxidants for the treatment of certain diseases heart disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract in mammals, particularly humans, processes for obtaining them and delivery formats therefore.

Brief description of the extract nomenclature:

Nomenclature of Plant extracts.



1. **AV**- first two letters represents **Avesthagen**.
2. Plant Name: The Plants used and in use are assigned with unique 3-digit number, **016** represents ***Terminalia arjuna***.
3. Part of the plant /Tissue: There is a two letter ID for each plant part used. For example **Ba** for **Bark**, **Fr** for **whole Fruit**.
4. Solvents: The solvents used for extraction are also assigned with two digit numbers **01** for **Acetone**, **02** for **Benzene**, **03** for **Chloroform**, **04** for **Ethanol**, **05** for **Hexane**, **06** for **Methanol**, **07** for **Petroleum ether**, **08** for **water**, **09** for **ethyl acetate**. **Percentage of solvents** used for extraction is given within **bracket** (20) for 20 % of that solvent. For example if 20% of Ethanol was used for extraction, 04(20).
5. Method of Extraction: **Successive extraction** is referred to as **Su** whereas **direct extraction** is referred to as **Di**, **temperature for extraction** is written in **bracket**. For example, Su(65) represents successive extraction at 65 °C.
6. Type of extract, g: gluey and ng: non-gluey.

Brief Description of the Tables and Figures:

Table 1: HPLC fingerprint of the extract AV016BaDi(65)04(100).

Table 2: HPLC fingerprint of the extract AV016BaDi(28)04(20).

Detailed description of the invention

In a first aspect of the invention there is provided a method for treating a disease in a mammal, which comprises administering to the said mammal an effective non-toxic amount of at least an extract from *Terminalia arjuna* as defined herein. Preferably the mammal is a human being. The skilled addressee will appreciate that "treating a disease" in a mammal means treating, that is to say, alleviating symptoms of the disease and may also mean managing a disease in the sense of preventing such a disease state either advancing ie getting worse or becoming more invasive, or slowing down the rate of advance of a disease.

In a second aspect of the invention, there is a provided a prophylactic method for preventing the occurrence of a disease state in a mammal which comprises administering to the said mammal an effective non-toxic amount of an extract from *Terminalia arjuna* as defined herein in the preparation of a comestible (=foodstuff) for prophylaxis against the occurrence of a disease diseases like cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract. Preferably the mammal is human and the said extract comprises a single extract from a plant part of Terminalia arjuna or a combination of extracts therefrom as detailed herein. Thus the present invention further relates to extracts which may be isolated from different parts of the *Terminalia arjuna* plant such as the bark and fruit thereof, the preparation of such extracts, medicaments comprising such extracts, and the use of these extracts and constituents for the preparation of a medicament.

Extracts of the present invention can be isolated from Terminalia tree species, such as *Terminalia arjuna,* using conventional organic solvent extraction and

supercritical fluid extraction technology. Generally, extracts of the invention capable of functioning in a prophylactic or therapeutic manner as outlined herein can be extracted from any *Terminalia arjuna* plant tissue, such as bark or fruit, depending on the end purpose that is required of the extract.

In a third aspect of the present invention there is provided a process for preparing extracts of the invention from plant parts of *Terminalia arjuna* that comprises:

- Pulverizing selected plant material to a powder;
- Subjecting the powdered plant material to solvent extraction;
- Lyophilizing the obtained extracts.

The choice of selected plant material may be of any type but is preferably selected from the bark or the fruit of the *Terminalia arjuna* plant.

The solvent extraction process may be selected from direct or successive extraction types such as extraction from plant parts in soxhlet apparatus or in flasks at room temperature or at higher temperature with polar and/or non-polar solvent(s). Typically, the extraction process is as outlined herein.

It will be apparent to the skilled addressee that the selection of solvent, or mixtures of solvents for each step in the isolation of extracts of the invention showing activity can be guided by results of bioassay analysis of separate fractions, for example as indicated herein and/or as shown in the examples.

Also encompassed within the ambit of the invention is a pharmaceutical formulation suitable for use in the treatment of a disease selected from the group heart disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; comprising at least one extract

as isolated from a *Terminalia* species, such as *Terminalia arjuna*, in admixture with a pharmaceutically acceptable carrier. Preferably, the at least one extract is selected from those listed in Tables 1 – 24 inclusive, depending on design and disease of interest. Preferably the at least one extract is selected from the group of extracts as defined in Tables 25 – 27 inclusive, again depending on end purpose. Naturally, the skilled addressee will appreciate that such compositions may comprise of two or more plant extracts of the invention in any concentration, which is capable of giving rise to a therapeutic effect. Thus, therapeutic compositions can comprise plant extracts of *Terminalia* substantially devoid of undesirable contaminating compounds. The plant extracts may have, for example, undergone a number of solvent extraction steps substantially to separate out undesirable components from desirable components such as those alluded to in the examples and aforementioned tables.

The invention thus further provides a method for the treatment of a disease selected from the group heart disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; in mammals, including humans, which comprises the use of a clinically useful amount of an extract selected from those listed in Tables 1 – 24 inclusive, preferably those listed in Tables 25 – 27 inclusive, in a pharmaceutically useful form, once or several times a day or in any other appropriate schedule for example, orally, or intravenously or by delivery to the lungs in a dry or "wet" spray.

The amount of compound of extract required to be effective in the treatment of the aforementioned diseases will, of course, vary with the disease being treated and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be considered include the condition being treated, the route of administration, and nature of the formulation, the mammal's body weight, surface area, age and general condition and the particular compound to be administered. A suitable

effective dose of an extract of the invention generally lies in the range of about 0.01 to about 120 mg/kg bodyweight, e.g. 0.1 to about 120 mg/kg body weight, preferably in the range of about 0.1 to 50 mg/kg, for example 0.5 to 50 mg/kg. The total daily dose may be given as a single dose, multiple doses, e.g. two to six times applications per day. For example, for a 75 kg mammal (e.g. a human) the dose range would be about 8 to 9000 mg per day, and a typical dose could be about 50 mg per day. If discrete multiple doses are indicated treatment might typically be 15 mg of a compound of Formula (I) given up to 4 times per day.

Whilst it is possible for the active extract to be administered alone, it is preferred to present the active extract in a pharmaceutical formulation. Formulations of the present invention, for medical use, comprise an extract of the invention together with one or more pharmaceutically acceptable carriers and optionally other therapeutic ingredients. The carrier(s) should be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and substantially non-deleterious to the recipient thereof.

The present invention, therefore, further provides a pharmaceutical formulation comprising at least one extract selected from those listed in tables 1 – 24 inclusive, preferably from those mentioned in tables 25 – 27 inclusive together with a pharmaceutically acceptable carrier therefore. In a preferment the pharmaceutical formulation comprises at least an extract selected from those listed in tables 25–27, depending on the disease type being treated. Naturally, the skilled addressee will appreciate that when selecting more than one extract from those given in the aforementioned tables for the treatment of any single disease type, that an appropriate selection of extracts fro the disease type will be made. Thus, for example, for the treatment of diabetes, extracts appropriate for doing so will be selected from the said tables.

Naturally, the skilled addressee will appreciate that any pharmaceutical formulation comprising an active extract of the invention can include at least one active extract purified from an extract derived from a *Terminalia* species. Thus a

pharmaceutical formulation may contain more than one active extract derived from two or more *Terminalia* species.

There is also provided a method for the preparation of a pharmaceutical formulation comprising bringing into association an extract of the invention, and a pharmaceutically acceptable carrier therefore.

Formulations according to the present invention include those suitable for oral or intravenous administration.

Intravenous formulations including at least one extract of the invention and may also be administered in the form of suitable liposomal or niosomal preparations or other suitable delivery vehicle.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween 60, Span 80, cetostearyl alcohol, myristyl alcohol, glycerol mono-stearate and sodium laury sulphate.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active extracts(s) into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active extract(s) into association with a liquid carrier or a finely divided solid carrier or both and then, if necessary, shaping the product into desired formulations.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets, tablets, lozenges, comprising the active ingredient in a flavoured based, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier. Each formulation generally contains a predetermined amount of the active extract; as a powder or granules; or a solution

or suspension in an aqueous or non-aqueous liquid such as a syrup, an elixir, an emulsion or draught and the like.

A tablet may be made by compression or moulding optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing an a suitable machine the active extract in a free-flowing form such as a powder or granules, optionally mixed with a binder, (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered extract moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide the desired release profile.

A syrup may be made by adding the active extract to a concentrated, aqueous solution of a sugar, for example sucrose, to which may also be added any necessary ingredients. Such accessory ingredient(s) may include flavourings, an agent to retard crystallisation of the sugar or an agent to increase the solubility of any other ingredients, such as a polyhydric alcohol for example glycerol or sorbitol.

In addition to the aforementioned ingredients, the formulations of this invention may further include on or more accessory ingredients) selected from diluents, buffers, flavouring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like.

Alternatively, the compositions are dietary supplements, food compositions or beverage compositions suitable for human or animal consumption.

The invention describes the HPLC profiles and Mass spectrums of direct and successive solvent extracts of Terminalia arjuna plant parts thereby giving each

extract an identity of itself. The various solvents used for successive extraction are in order from non-polar to polar side i.e hexane, petroleum ether, ethyl acetate, acetone, ethanol, methanol and water. In case of direct extraction alcoholic solvent alone and in combination with water was used as solvent for extraction.

The invention further encompasses novel extracts defined by reference to their HPLC and MS fingerprints as defined in Tables 1 – 24 inclusive, which are isolated from different parts of Terminalia arjuna plant, the preparation of such extracts, the medicaments containing said extracts, and the use of these extracts and constituents for the preparation of a medicament.

In one embodiment of the invention, the compositions for preventing, treating, or managing diseases such as heart disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract comprises of direct extracts of *T. arjuna* bark with 100% ethanol solvent [AV016BaDi(65)04(100)] and 20% ethanol solvent [AV016BaDi(28)04(20)], successive extract of *T. arjuna* bark with ethyl acetate solvent [AV016BaSu(65)09(100)], successive extract of *T. arjuna* bark with acetone solvent [AV016BaSu(65)01(100)], [AV016BaSu(65)01(100)g] and [AV016BaSu(65)01(100)ng], successive extract of *T. arjuna* bark with ethanol solvent [AV016BaSu(65)04(100)], successive extract of *T. arjuna* bark with methanol solvent [AV016BaSu(65)06(100)] and successive extract of *T. arjuna* bark with water solvent [AV016BaSu(105)08(100)], direct extract of *T. arjuna* fruit with ethanol solvent [AV016FrDi(65)04(100)] and successive extract of *T. arjuna* fruit with water solvent [AV016FrSu(105)08(100)], alone or in combination thereof. The compositions/medicaments may contain a pharmaceutically acceptable carrier, excipient, or diluent.

In another embodiment of the invention, the compositions for preventing, treating, or managing microbial infections comprises of successive extract of *T. arjuna* bark with ethyl acetate solvent [AV016BaSu(65)09(100)], successive extract of *T. arjuna* bark with acetone solvent [AV016BaSu(65)01(100)], successive extract of *T. arjuna* bark with ethanol solvent [AV016BaSu(65)04(100)], successive extract of *T. arjuna* bark with methanol solvent [AV016BaSu(65)06(100)] and successive extract of T. arjuna bark with water solvent [AV016BaSu(105)08(100)], direct extract of *T. arjuna* fruit with ethanol solvent [AV016FrDi(65)04(100)] and successive extract of *T. arjuna* fruit with water solvent [AV016FrSu(105)08(100)], alone or in combination thereof. The compositions/medicaments may contain a pharmaceutically acceptable carrier, excipient, or diluent.

In a further aspect of the invention there is provided a comestible, that is to say, a foodstuff comprising at least an extract of the invention, typically in dried form, such as in a lyophilized form selected from those listed in Tables 1 – 24 herein, and in particular, from those extracts selected from those mentioned in Tables 25 – 27. The skilled addressee will appreciate that such comestibles may contain more than one extract of the invention and may be used. Such foodstuffs may be used in a prophylactic manner and may contain further extracts having a similar function to the first added extract or further added extracts may be added that have a different prophylactic function. Thus a foodstuff could either comprise extracts that provide for a comestible having a single functional aspect, for example that of having a prophylactic effect against the occurrence of diabetes, or a comestible may have a multi-functional prophylactic effect against two or more disease types. It is thought that a similar multi-functional role could also be assigned to pharmaceutical formulations comprising two or more extracts possessing dissimilar therapeutic or prophylactic properties designed either for prophylaxis or for the treatment of more than one disease(s) in a mammal, particularly in a human.

The type of foodstuff or comestible to which at least an extract of the invention may be added includes any processed food such as confectionaries, baked products including breads such as loafs, and flat breads such as pitta bread, naan bread and the like, cakes, snack foods such as muesli bars, compressed dried fruit bars, biscuits, dairy products such as yoghurts, milk and milk-based products such as custards, cream, cheese, butter and crème fraiche, simulated dairy food products such as margarine, olive oil-based spreads, and low fat cream substitutes such as Elmlea products, fruit and vegetable juices, aerated drinks, such as carbonated soft drinks and non-aerated drinks such as squashes, soya milk, rice milk and coconut milk and the like, pastas, noodles, vegetable, seed and nut oils, fruited oils such as sunflower oil, rapeseed oil, olive oil, walnut, hazelnut, and sesame seed oil and the like, and frozen confections such as ice creams, iced yoghurts and the like.

A suitable effective dose of an extract of the invention to be included in a comestible generally lies in the range of about 0.01 to about 120 mg/kg bodyweight, e.g. 0.1 to about 120 mg/kg body weight, preferably in the range of about 0.1 to 50 mg/kg, for example 0.5 to 50 mg/kg. The total daily dose may be given as a single dose, multiple doses, e.g. two to six times applications per day.

In a further aspect of the invention there is provided use of an extract selected from those of Tables 1 – 24, and in particular those of Tables 25 – 30 for the preparation of a medicament for the treatment of a disease selected from the group consisting of heart disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract.

Thus, there is provided use of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng,

AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), alone or in combination thereof for the preparation of a medicament for the treatment or prophylaxis of such as cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart diseases, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; owing to the extracts antioxidation potential.

Thus, there is provided use of an extract selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100)] and AV016FrSu(105)08(100)], alone or in combination thereof for the preparation of a medicament for the treatment or prophylaxis of infectious diseases, owing to the extracts antimicrobial potential.

The invention will now be exemplified with reference to the following Examples section and accompanying tables and Figures. It is to be understood that the examples are not to be construed as limiting the scope of the invention in any way.

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Examples Section

Example 1 : Extraction of *Terminalia arjuna*:

Extraction of *Terminalia arjuna* plant parts was carried out by both direct extraction as well as successive extraction method, at room temperature as well as in soxhlet apparatus and related liquid-liquid techniques followed by lyophilization.

I. Successive Extraction:

Successive extraction from bark of *Terminalia arjuna* was carried out using soxhlet extractor. The solvents used, were based on their sequential polarity starting from non-polar to polar, viz; Hexane, chloroform, ethyl acetate, acetone, ethanol, methanol and water at 65°C / above boiling point of the solvent.

The detailed process is given below:

1. Weigh 50 grams of powdered plant material into the extractor (Soxhlet extractor body) with the cotton on the bottom of the soxhlet apparatus. Cover with cotton on the top. Add 250 ml of solvent (start with Hexane) in to the round-bottomed flask and place it on the mantle and add few ceramic chips in to it. Add 50ml of solvent over the material just wetting it.
2. Place the extractor on the flask, which is in turn connected with the condenser.
3. Let the cold water circulate continuously in the condenser from the tap. The set up fits tightly as it is fabricated as one set.
4. Switch on the mantle and set it at 65 °C. The vapors of the solvent from the flask would pass through the inlet of the extractor and will get

condensed. The condensed (distilled) Solvent will get collected in the Extractor (body) thus extracting the compounds from it.

5. When the plant material is completely filled with solvents, it will get drained in the flask. This process is continuous as long as there is stable heat and water circulation.
6. Continue the extraction for 8 hours, 4-5 cycles per hour.
7. The extract collected in the flask is concentrated by vacuum lyophilization.
8. Follow the same procedure as above successively for the following solvents in the same order. Hexane, chloroform, Ethyl acetate, Acetone, Methanol and Water.

II. Direct Extraction

a. Soxhlet based extraction procedure with 100% ethanol solvent:

1. Weigh 100 grams of powdered plant material in the cloth bag and transfer it into the extractor (Soxhlet extractor body). Cover with cotton on the top. (Make sure the level of material is below one inch of the vapour inlet tube.)
2. Add 1 liter of solvent (start with Pet. ether) in to the round-bottomed flask and place it on the mantle and add few ceramic chips in to it. Add 100ml of solvent over the material just wetting it.
3. Place the extractor on the flask, which is in turn connected with the condenser.
4. Let the cold water circulate continuously in the condenser from the tap. The set up fits tightly as it is fabricated as one set.
5. Switch on the mantle and set it at 65 °C. The vapours of the solvent from the flask would pass through the inlet of the extractor and will get condensed. The condensed (distilled) Solvent will get collected in the Extractor (body) thus extracting the compounds from it.

6. When the plant material is completely filled with solvents, it will get drained in the flask. This process is continuous as long as there is stable heat and water circulation.
7. Continue the extraction for 8 hours, 4-5 cycles per hour.
8. The extract collected in the flask is concentrated by vacuum lyophilization.

b. Extraction of T. arjuna bark with 20% ethanol solvent at room temperature:

1. Weigh known quantity (100 grams) of powdered plant material into the conical flask and cover the mouth with aluminum foil to avoid solvent evaporation.
2. Add known volume (500 ml) of 20 % ethanol (100 ml ethanol + 400 ml water) solvent in to the flask and place it on to the orbital shaker and set the speed at 210 rpm and room 28 °C temperature for the extraction.
3. Extract the plant material for 4hr and drain the solvent through
4. Centrifuge the filtrate at 1000 rpm for 10 mins. Collect the supernatant and subject it to lyophilization.
5. Re-extract with 250 ml of solvent for (2 x 2hrs).
6. Centrifuge the filtrate at 1000 rpm for 10 mins.
7. Concentrate extract using lyophilizer under vacuum.

Example 2: Metabolic Fingerprinting of the *Terminalia arjuna* extracts:

Metabolic fingerprinting of all the direct and successive extracts from *Terminalia arjuna* plant parts is done by HPLC and LC-MS technique.

I. HPLC Fingerprinting:

The plant extracts obtained by direct/successive extraction are subjected to HPLC analysis. High Performance Liquid Chromatography (HPLC) is a technique

wherein small quantity of the sample is injected into a C-18 column under high pressure and the constituents are allowed to separate based on their interaction with the column and their retention time within the column. The main purpose of HPLC analysis is to find out the total number of constituents in the plant extracts.

The samples are prepared for HPLC analysis by dissolving the appropriate weight of the extract in methanol. These samples are filtered and collected in the total recovery HPLC vials. These samples are subjected to separation by Waters 2695 HPLC instrument and then analyzed at 250 nm.

Run conditions:

1. The software used for HPLC analysis is Waters Millennium[32].
2. The HPLC column used for separation is Waters μBondpack C-18, 5μ, 4.6x150mm.
3. Column temperature is maintained at 25^0C.
4. Solvent flow rate is set at 1.0ml per min. HPLC conditions included Gradient chromatography - solvents used are acetonitrile (solvent A), methanol (solvent B) and water (Solvent C and D).

***Terminalia arjuna* extracts and HPLC Run Conditions:**

1. *Terminalia arjuna* extracts:

1. AV016BaDi(65)04(100)
2. AV016BaDi(28)04(20)
3. AV016BaSu(65)04(100)
4. AV016FrDi(65)04(100)
5. AV016BaSu(65)06(100)

I. **Method Set: Ethanol_11**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	1.00	10.0	0.0	0.0	90.0	6
2	1.00	1.00	10.0	0.0	0.0	90.0	6
3	15.00	1.00	30.0	0.0	0.0	70.0	6
4	30.00	1.00	40.0	0.0	0.0	60.0	6

A: Acetonitrile, B: Methanol, C: Water

2. *Terminalia arjuna* extracts

1. AV016BaSu(65)01(100)
2. AV016BaSu(65)01(100)g
3. AV016BaSu(65)01(100)g

II. Method Set: Ethyl Acetate_10a

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	5.0	2.5	92.5	0.0	6

2	1.00	0.75	5.0	2.5	92.5	0.0	6
3	25.00	0.75	25.0	2.5	72.5	0.0	6
4	30.00	0.75	5.0	2.5	92.5	0.0	1

A: Acetonitrile, B: Methanol, C: Water

3. *Terminalia arjuna* extract:

1. AV016BaSu(65)09(100)

III. **Method Set: Ethyl Acetate_4a**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	0.0	5.0	95.0	0.0	6
2	1.00	0.75	0.0	5.0	95.0	0.0	6
3	15.00	0.75	0.0	20.0	80.0	0.0	6
4	25.00	0.75	0.0	50.0	50.0	0.0	6
5	30.00	0.75	0.0	5.0	95.0	0.0	1

A: Acetonitrile, B: Methanol, C: Water

4. *Terminalia arjuna* extract:

1. AV016BaSu(105)08(100)
2. AV016FrSu(105)08(100)

IV. **Method Set: Gy_Water_12**

Pressure Limits:

High Limits 4000 psi Low limits 0 psi

Programmed Flow:

Pump Mode: Gradient

Accelerated to 10 ml/min in: 2.0 min (5ml/min/min)

	Time	Flow	%A	%B	%C	%D	Curve
1	0.01	0.75	5.0	5.0	90.0	0.0	6
2	1.00	0.75	5.0	5.0	90.0	0.0	6
3	20.00	0.75	25.0	15.0	60.0	0.0	4
4	26.00	0.75	70.0	5.0	25.0	0.0	4
5	30.00	0.75	5.0	5.0	90.0	0.0	1

A: Acetonitrile, B: Methanol, C: Water

II. LC/MS Fingerprinting:

II. Liquid Chromatography Mass Spectrometry (LC/MS) Fingerprinting:

Mass spectroscopy, is an instrumental approach that allows for the mass measurement of molecules. The five basic components of mass spectrometer are a vacuum system, a sample introduction device, an ionization source, a mass analyzer and an ion detector. Combining these parts a mass spectrometer determines the molecular weight of chemical compounds by ionizing, separating and measuring molecular ions according to their mass-to-charge ratio (m/z).

Run conditions used for LC/MS fingerprinting of *Terminalia arjuna* is shown down.

1. Q-Trap LC/MS instrument from Applied Biosystems was used. The software used for LC/MS analysis is Analyst.
2. The HPLC column used for separation is COSMOSIL® $5C_{18}$-MS-II Packed Column C-18, 5μm, 4.6mmI.D.x 150mm.
3. Column temperature is maintained at 25^0C.
4. Solvent flow rate is set at 1.0ml per min. HPLC conditions included Gradient chromatography - solvents used are acetonitrile (solvent C), methanol (solvent B) and water (Solvent D).

1. *Terminalia arjuna* extracts:

AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)g, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaDi(65)04(100), and AV016FrDi(65)04(100)

a. LC/MS Sample Run Conditions for all the above-mentioned *Terminalia arjuna* samples:

Mass Spectrometer SPEC	QTrap	0	MASS
Config Table Ver	01		
Firmware Ver	M401400 B4T0301 M3L1400 B3T0300		
Component Name	Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer		
Component ID	QTrap		
Manufacturer	AB Sciex Instruments		
Model	027170 - C		
S/N	M1100301		

Time from start =0.0500 min

Mass Spectrometer	QTrap	0	MASS
SPEC			
Start of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		0.7	
Backing Pump		Ok	
Dual Turbo Pump		Normal	
Sample Introduction Status		Ready	
Source/Ion Path Electronics		On	
Source Type		Turbo Spray	
Source Temperature (at setpoint)		400.0 C	
Source Exhaust Pump		Ok	
Interface Heater		Ready	

Mass Spectrometer	QTrap	0	MASS
SPEC			
End of Run - Detailed Status			
Vacuum Status		At Pressure	
Vacuum Gauge (10e-5 Torr)		0.7	
Backing Pump		Ok	
Dual Turbo Pump		Normal	
Sample Introduction Status		Ready	
Source/Ion Path Electronics		On	
Source Type		Turbo Spray	
Source Temperature (at setpoint)		400.0 C	
Source Exhaust Pump		Ok	
Interface Heater		Ready	
Time from start =61.4833 min			

PE LC-200 Pump Method Properties

PE LC-200 Quaternary Pump

Minimum Pressure (psi):0.0

Maximum Pressure (psi): 6100.0

Shutdown Time (min): 999.9

Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	GradientProfile	A (%)	B (%)	C (%)	D (%)	TE#1	TE#2
0	0.5	1000.00	1.0	0.0	0.0	10.0	90.0	open	open
1	1.0	1000.00	1.0	0.0	0.0	10.0	90.0	open	open
2	15.0	1000.00	1.0	0.0	0.0	15.0	85.0	open	open
3	40.0	1000.00	1.0	0.0	0.0	25.0	75.0	open	open
4	50.0	1000.00	1.0	0.0	0.0	35.0	65.0	open	open
5	60.0	1000.00	1.0	0.0	0.0	50.0	50.0	open	open

Quantitation Information:

Sample Type: Unknown

Dilution Factor: 1.000000

Custom Data:

Quantitation Table:

Period 1:

Scans in Period: 2243

Relative Start Time: 0.00 msec

Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: Q1 MS (Q1)

Polarity: Positive

Scan Mode: Profile

Resolution Q1:	UNIT
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 ms
MR Pause:	5.0070 ms
MCA:	No
Center/Width:	No
Step Size:	0.10 amu

Start (amu)	Stop (amu)	Time (sec)	Param	Start	Stop
50.00	1700.00	1.60	CEP	6.47	66.65

Parameter Table(Period 1 Experiment 1)

CUR:	20.00
TEM:	400.00
GS1:	20.00
GS2:	50.00
ihe:	ON
IS:	4500.00
DP	90.00
EP	10.00

2. *Terminalia arjuna* extracts:

AV016BaSu(105)08(100), AV016FrSu(105)08(100) and AV016BaDi(28)04(20).

a. LC/MS Sample Run Conditions for all the above-mentioned *Terminalia arjuna* samples:

Mass Spectrometer	QTrap	0	MASS SPEC
Config Table Ver	01		
Firmware Ver	M401400 B4T0301 M3L1400 B3T0300		

Component Name	Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer
Component ID	QTrap
Manufacturer	AB Sciex Instruments
Model	027170 - C
S/N	M1100301

Time from start =2.1000 min

Mass Spectrometer	QTrap	0	MASS SPEC

Start of Run - Detailed Status	
Vacuum Status	At Pressure
Vacuum Gauge (10e-5 Torr)	0.7
Backing Pump	Ok
Dual Turbo Pump	Normal
Sample Introduction Status	Ready
Source/Ion Path Electronics	On
Source Type	Turbo Spray
Source Temperature (at setpoint)	400.0 C
Source Exhaust Pump	Ok
Interface Heater	Ready

Time from start =2.1167 min

Mass Spectrometer	QTrap	0	MASS SPEC

End of Run - Detailed Status	
Vacuum Status	At Pressure
Vacuum Gauge (10e-5 Torr)	0.7
Backing Pump	Ok
Dual Turbo Pump	Normal

Sample Introduction Status	Ready
Source/Ion Path Electronics	On
Source Type	Turbo Spray
Source Temperature (at setpoint)	400.0 C
Source Exhaust Pump	Ok
Interface Heater	Ready

Time from start =42.9333 min

PE LC-200 Pump Method Properties

PE LC-200 Quaternary Pump

Minimum Pressure (psi):0.0

Maximum Pressure (psi): 6100.0

Shutdown Time (min): 999.9

Step Table:

Step	Total Time (min)	Flow Rate (µl/min)	GradientProfile	A (%)	B (%)	C (%)	D (%)	TE#1	TE#2
0	0.5	750.00	1.0	0.0	5.0	5.0	90.0	open	open
1	1.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open
2	20.0	750.00	1.0	0.0	15.0	25.0	60.0	open	open
3	26.0	750.00	1.0	0.0	5.0	70.0	25.0	open	open
4	30.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open
5	40.0	750.00	1.0	0.0	5.0	5.0	90.0	open	open

Analog/Digital Converter Properties

Interval (sec):	0.200
Rate (pts/sec):	5
Polarity:	Bipolar

Channel Summary

No.	Name:	Interpreted Value	Full Scale:	Interpreted Unit:
	Voltage (volts):	Status:		
1		100.0	%	1.0 Used

Quantitation Information:

Sample Type: Unknown
Dilution Factor: 1.000000

Custom Data:
Quantitation Table:

Period 1:

Scans in Period: 1495
Relative Start Time: 0.00 msec
Experiments in Period: 1

Period 1 Experiment 1:

Scan Type: Q1 MS (Q1)
Polarity: Positive
Scan Mode: Profile
Resolution Q1: UNIT
Intensity Thres.: 0.00 cps
Settling Time: 0.0000 ms
MR Pause: 5.0070 ms
MCA: No
Center/Width: No
Step Size: 0.10 amu

Start (amu)	Stop (amu)	Time (sec)	Param	Start	Stop
50.00	1700.00	1.60	CEP	6.47	66.65

Parameter Table(Period 1 Experiment 1)

CUR:	20.00
TEM:	400.00
GS1:	20.00
GS2:	50.00
ihe:	ON
IS:	4500.00
DP	90.00
EP	10.00

Example 3: Determination of the bio-therapeutic potential of *Terminalia arjuna* extracts:

A. Antioxidant assay:

The antioxidant activities of natural components may have reciprocal correlation with their reducing potentials. Several methods have been developed to measure the efficacy of dietary antioxidants as pure compounds or in food extracts. These methods focus on different mechanisms of the oxidant defense system i.e. scavenging active oxygen species and hydroxyl radicals, reduction of lipid peroxyl radicals, inhibition of lipid per-oxidation, or chelation of metal ions. In most of the cases irrespective of the stage in the non-enzymatic anti-oxidative activity (scavenging of free radicals, inhibition of lipid per-oxidation, etc.) is mediated by redox reactions.

1. DPPH Scavenging Effect

a. Assay Principle

This method is based on the reduction of DPPH, a stable free radical. Due to the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). As the odd electron of the radical becomes paired off in the presence of hydrogen donor, that is, a free-radical scavenging antioxidant, the absorption strength is decreased, and the resulting de-coloration is stoichiometric with respect to the number of electrons captured. This reaction has widely been used to evaluate the anti-oxidative activity of food and plant extracts.

b. Assay method

Reactions were performed in 1.25 ml of methanol containing 0.5 mM freshly made DPPH and various amounts of the extract. Reaction mixtures were incubated at 37 °C for 30 min, and the absorbance at 517 nm was measured. This assay was done in triplicate.

Oxidant (DPPH) inhibitory activity (%) = $\{(A_{517}\text{Control} - A_{517}\text{Sample})/A_{517}\text{Control}\} \times 100$

c. Results and Discussions:

It was found that the reduction of DPPH radical was dose dependent. IC_{50} is defined as the amount of extract required for 50% inhibition in the levels of free radical. Table 25 gives the IC_{50} values of *Terminalia arjuna* bark and fruit extracts.

IC_{50} of *Terminalia arjuna* successive extracts AV016BaSu(65)01(100), AV016BaSu(65)09(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016BaSu(65)01(100)g and

AV016BaSu(65)01(100)ng was determined as 25.0 μg/ml , 52.8 μg/ml, 36.8 μg/ml, 34.3 μg/ml, 46.4 μg/ml, 26 μg/ml and 46 μg/ml respectively (Fig 1 and 2).

In case of *T. arjuna* fruit ethanol AV016FrDi(65)04(100) extract and water extract AV016FrSu(105)08(100) was found to be 34 μg/ml and 39 μg/ml respectively (Fig 3).

Inhibitory concentration (IC_{50} values) of *Terminalia arjuna* direct bark 100% ethanol extract AV016BaDi(65)04(100) was found to be 26 μg/ml whereas that of 20% direct ethanol extract AV016BaDi(28)04(20) was found to be 24 μg/ml (Fig 4 and 5).

Conclusions

It was seen that IC50 of AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaDi(65)04(100) and AV016BaDi(28)04(20) extracts was found to be less than that of ascorbic acid, thereby showing potential anti-oxidation potential.

B. Antibacterial assay:

Cultures tested:

Testing of anti-microbial potential was done against following bacterial strains (Gram negative: *Escherichia coli* ATCC-10536, *Pseudomonas aeruginosa* ATCC-9027, *Klebsiella pneumoniea* ATCC-10031, *Bordetella bronchiseptica* ATCC-4617; Gram Positive: *Staphylococcus aureus* ATCC-29737, *Streptococcus fecalis* ATCC-8043, *Micrococcus luteus* ATCC-9341, *Bacillus subtilis* ATCC-6633, *Bacillus cereus* ATCC-11778, *Bacillus pumilus* ATCC-14884, *Staphylococcus epidermidis* ATCC-6358) were selected from the microorganisms

given in United states Pharmacopoeia (2000), British Pharmacopoeia (1993) and Indian Pharmacopoeia (1996) for anti-microbial assays.

Agar streak method:

A stock of 100 mg/ml of Ethyl acetate, Acetone, Ethanol, Methanol and Water successive extract from *Terminalia arjuna* bark and direct ethanol and successive water extracts from *Terminalia arjuna* fruit was dissolved in DMSO. To determine the antibacterial potential extracts at a concentration of 5 mg/ml and 1 mg/ml were added to 30 ml of luke warm Luria Bertaini agar medium. After the medium was solidified, overnight grown 11 bacterial strains mentioned were taken in loop and streaked on the medium. The plates were incubated at 37 °C for 24 hrs after which the bacterial growth was monitored. Suitable controls were maintained with the extracts and the microorganisms. Luria Bertaini agar medium with and without 1.5 % DMSO were used as negative control set, Ciprofloxacin (2 μg/ml) served as positive control.

Results and Discussion:

Table 2 and 3 enumerates the antibacterial properties of *Terminalia arjuna* plant part extracts against the standard ATCC bacterial stains used for testing the anti-bacterial potential of the test compounds.

It is observed that at concentration of 5 mg/ml AV016BaSu(65)09(100) extract exhibited a broad antibacterial inhibiting growth of 9 of the 11 bacterial strains tested (Fig 6). The extract was found to be very effective against the gram-positive bacteria showing inhibition of all the seven gram positive strains tested. Whereas AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100) and AV016BaSu(105)08(100) extract showed inhibition against *B. bronchiseptica, S. aureus, S. fecalis* and *M. luteus* (Fig 7).

At concentration of 1 mg/ml AV016BaSu(65)09(100) extract showed antibacterial activity against *B. bronchiseptica, S. aureus* and *S. fecalis*. AV016BaSu(65)01(100) extract showed complete inhibition of growth of *S. aureus* and *S. fecalis* whereas showed partial growth inhibition against *B. bronchiseptica.* AV016BaSu(65)04(100),AV016BaSu(65)06(100) and AV016BaSu(105)08(100) extract showed inhibition against only *S. aureus.*

Terminalia arjuna direct ethanol fruit extract AV016FrDi(65)04(100) also showed wide spectrum anti-bacterial activity. AV016FrDi(65)04(100) extract at concentration of 5mg/ml showed bacteriostatic effect against the test strains *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniea, Staphylococcus aureus, Streptococcus fecalis and Micrococcus luteus.* AV016FrDi(65)04(100) extract showed completed inhibition of the test stains *Bordetella bronchiseptica, Bacillus cereus, Bacillus pumilus* and *Staphylococcus epidermidis* at concentration of 5 mg/ml. At concentration of 1 mg/ml AV016FrDi(65)04(100) extract extract showed completed inhibition of *Bordetella bronchiseptica.*

AV016FrSu(105)08(100) extract at concentration of 5 mg/ml showed inhibitory effect only against *Bordetella bronchiseptica.*

Table 1: HPLC fingerprint of extract AV016BaDi(65)04(100)

	Retention Time	Area	% Area	Height
1	1.746	83501	0.03	18746
2	1.887	21939706	9.17	3508953
3	2.482	424664	0.18	27549
4	3.165	694426	0.29	38196
5	4.499	535430	0.22	20883
6	5.860	1140766	0.48	31447
7	6.488	2287279	0.96	105829
8	7.680	2247351	0.94	73739
9	8.259	1172094	0.49	53148
10	8.702	2209397	0.92	93831
11	9.425	2269048	0.95	89553
12	10.177	3848206	1.61	111334
13	10.485	1536103	0.64	99241
14	10.730	1306318	0.55	102448
15	11.155	3705535	1.55	129030
16	12.263	10146875	4.24	464227
17	12.335	6747979	2.82	548102
18	13.000	30474340	12.74	1778085
19	13.697	3437393	1.44	233361
20	14.232	8223596	3.44	277375
21	14.532	14638851	6.12	819721
22	15.023	12657916	5.29	590365
23	15.452	5471266	2.29	306520
24	15.998	16599596	6.94	1078704
25	16.299	4397452	1.84	318952
26	16.463	2424066	1.01	310668
27	16.863	6414264	2.68	310396
28	17.166	12652492	5.29	355032
29	17.683	4940680	2.07	294832
30	18.118	11457746	4.79	318670
31	18.673	2951869	1.23	232444
32	18.872	6643592	2.78	219376
33	19.475	1933618	0.81	176183
34	19.668	2454443	1.03	182659
35	19.884	4858876	2.03	167366
36	20.329	950214	0.40	139162
37	20.543	6254360	2.61	134329
38	21.427	1804592	0.75	107259
39	21.716	7198552	3.01	109172
40	23.185	2885830	1.21	72715
41	23.905	3042057	1.27	54167
42	24.977	665797	0.28	38055
43	25.258	1486354	0.62	35170

Table 2: HPLC fingerprint of extract AV016BaDi(28)04(20).

	Retention Time	Area	% Area	Height
1	1.724	259750	0.10	58489
2	1.897	27826788	10.53	3799266
3	2.486	571316	0.22	50306
4	3.045	1106233	0.42	51910
5	3.732	674291	0.26	29021
6	4.182	1056425	0.40	32454
7	5.572	2403677	0.91	47788
8	6.082	3557804	1.35	161895
9	6.484	757991	0.29	55308
10	7.264	3549212	1.34	97606
11	7.883	2512425	0.95	92337
12	8.299	3676625	1.39	140954
13	9.060	4300244	1.63	140449
14	9.699	6186918	2.34	155916
15	10.124	2085208	0.79	151077
16	10.796	7036369	2.66	179944
17	11.555	17109248	6.48	350960
18	12.574	31568344	11.95	1286226
19	13.038	2562494	0.97	285400
20	13.307	4871494	1.84	288682
21	14.002	26201944	9.92	838854
22	14.597	14156853	5.36	465017
23	15.150	4497591	1.70	322199
24	15.663	16422598	6.22	962872
25	15.912	3863537	1.46	326789
26	16.103	6416104	2.43	315587
27	16.564	4817209	1.82	305649
28	16.767	8560395	3.24	305526
29	17.221	2824887	1.07	283965
30	17.400	2813123	1.06	282612
31	17.661	16467191	6.23	382391
32	18.625	6447644	2.44	200677
33	19.302	3348258	1.27	168434
34	19.568	11717199	4.44	149595
35	21.405	4945619	1.87	87932
36	22.629	4393424	1.66	69440
37	24.162	2305522	0.87	34152
38	25.963	255400	0.10	9559

Table 3: HPLC fingerprint of extract AV016BaSu(65)09(100).

	Retention Time	Area	% Area	Height
1	2.475	25395907	5.13	3334075
2	3.244	1688929	0.34	136464
3	4.656	9262251	1.87	280029
4	5.254	1188708	0.24	48754
5	6.603	12296585	2.48	364675
6	6.899	5848912	1.18	389909
7	7.233	1367772	0.28	130776
8	7.428	1332175	0.27	138611
9	7.896	7084034	1.43	280115
10	8.260	6705156	1.35	380995
11	8.576	2924953	0.59	246136
12	8.782	2453519	0.50	228061
13	9.184	8669542	1.75	320819
14	9.521	4295969	0.87	304404
15	9.816	4279516	0.86	324956
16	10.243	30792046	6.22	3248747
17	10.516	84937402	17.16	4333685
18	11.442	12774541	2.58	1056539
19	11.603	33723533	6.81	4613519
20	11.705	63394649	12.81	4142892
21	12.316	44870739	9.06	3991785
22	12.575	5106719	1.03	582499
23	12.754	2812210	0.57	307827
24	12.983	7556632	1.53	836964
25	13.270	4609110	0.93	360754
26	13.418	4222758	0.85	346063
27	13.925	16253486	3.28	1431902
28	14.456	7163578	1.45	605726
29	14.797	1631474	0.33	141283
30	15.240	3874910	0.78	285386
31	15.388	3029615	0.61	225745
32	15.588	2719921	0.55	159504
33	16.139	1333368	0.27	123152
34	16.346	3614771	0.73	345601
35	16.570	1244982	0.25	111424
36	16.813	1256674	0.25	123386
37	16.995	1494705	0.30	110575
38	17.367	1278577	0.26	73925
39	17.823	2517030	0.51	117093
40	18.123	2322326	0.47	179787
41	18.419	841112	0.17	86329
42	18.673	2150256	0.43	93219
43	18.954	429553	0.09	73333
44	19.255	1852803	0.37	78786
45	19.627	1385755	0.28	84299
46	19.912	1175927	0.24	106435

47	20.169	2238619	0.45	198261
48	20.362	1903752	0.38	136402
49	20.725	1388092	0.28	86158
50	21.002	2038182	0.41	112055
51	21.221	957990	0.19	84615
52	21.507	1049496	0.21	79603
53	21.799	1772753	0.36	102185
54	22.203	1643957	0.33	76435
55	22.543	831012	0.17	71872
56	22.690	572833	0.12	72239
57	22.941	3535908	0.71	220985
58	23.299	2133293	0.43	142496
59	23.648	2144927	0.43	151460
60	23.960	1493722	0.30	100416
61	24.429	2297448	0.46	123572
62	24.639	908084	0.18	85150
63	24.933	2402551	0.49	104466
64	25.280	583388	0.12	83878
65	25.564	1925541	0.39	92355
66	25.800	1153719	0.23	90624
67	25.974	1727846	0.35	88759
68	26.404	1397501	0.28	91036
69	26.591	795071	0.16	89416
70	26.751	1482526	0.30	89528
71	27.018	763878	0.15	86118
72	27.157	1700407	0.34	86915
73	27.647	2637337	0.53	78919
74	28.193	4360949	0.88	662358

Table 4: HPLC fingerprint of extract AV016BaSu(65)01(100).

	Retention Time	Area	% Area	Height
1	2.391	6928	0.00	852
2	2.556	1739539	0.82	304280
3	3.313	84646	0.04	9680
4	3.686	223139	0.10	9176
5	4.269	63325	0.03	3419
6	5.024	13893	0.01	769
7	6.157	4281	0.00	572
8	6.736	197041	0.09	6916
9	7.391	117641	0.06	5898
10	7.878	616604	0.29	17813
11	8.308	278599	0.13	15829
12	8.860	611104	0.29	22047
13	9.391	187896	0.09	12237
14	9.564	276548	0.13	14167
15	12.058	8846367	4.16	183309
16	12.644	751247	0.35	38688
17	13.199	1719360	0.81	43466
18	13.988	1735451	0.82	56406
19	14.555	2169673	1.02	76948
20	14.875	2254289	1.06	115963
21	15.626	4155110	1.95	123796
22	15.956	1080279	0.51	86186
23	16.580	5912054	2.78	175797
24	17.766	7200465	3.38	141141
25	18.423	2986827	1.40	111890
26	19.067	6971841	3.28	151382
27	19.737	4017460	1.89	145309
28	20.104	1488984	0.70	136621
29	20.459	4173594	1.96	175505
30	20.795	3235535	1.52	167773
31	21.079	1788929	0.84	164753
32	21.272	4796914	2.25	175363
33	21.910	4124406	1.94	168234
34	22.267	6071222	2.85	185861
35	22.997	5930428	2.79	190259
36	23.451	5868309	2.76	231002
37	23.866	16287689	7.65	1076501
38	24.287	4035419	1.90	266032
39	24.627	3569391	1.68	266672
40	24.844	42015183	19.75	2046204
41	26.107	8757964	4.12	239370
42	26.900	25586681	12.02	821474
43	27.926	7429374	3.49	323025
44	28.156	13400123	6.30	654328

Table 5: HPLC fingerprint of extract AV016BaSu(65)01(100)ng.

	Retention Time	Area	% Area	Height
1	2.579	1411822	0.59	207187
2	3.336	80911	0.03	9988
3	3.518	367100	0.15	14428
4	4.289	54629	0.02	3130
5	4.766	46285	0.02	1682
6	6.659	58963	0.02	2489
7	7.316	3635	0.00	454
8	7.852	277194	0.12	10198
9	8.240	150308	0.06	8971
10	8.774	321493	0.14	13773
11	9.207	113505	0.05	7075
12	9.458	151700	0.06	8682
13	10.403	841535	0.35	23389
14	11.877	5609438	2.36	151067
15	12.469	548895	0.23	28503
16	12.997	1315579	0.55	34524
17	13.798	1396765	0.59	46557
18	14.301	1552115	0.65	56533
19	14.658	1693118	0.71	88234
20	15.378	2309177	0.97	76351
21	15.719	1849902	0.78	89567
22	16.341	3751422	1.58	131368
23	16.651	705622	0.30	59259
24	17.537	5824011	2.45	121156
25	18.170	2202916	0.93	92276
26	19.153	16377423	6.89	384476
27	20.514	11154160	4.69	206032
28	21.248	27468304	11.56	1141614
29	22.005	3503203	1.47	178231
30	22.665	5722669	2.41	184191
31	23.185	15337448	6.45	535651
32	23.666	13965998	5.88	926816
33	24.155	2815614	1.18	263179
34	24.360	4975496	2.09	287271
35	24.677	30074237	12.65	1853497
36	25.316	9050757	3.81	411682
37	25.712	6189308	2.60	264816
38	26.607	20990123	8.83	826640
39	26.996	4842260	2.04	310728
40	27.520	16829287	7.08	814850
41	27.942	15732031	6.62	542909

Table 6: HPLC fingerprint of extract AV016BaSu(65)01(100)g.

	Retention Time	Area	% Area	Height
1	2.384	12028	0.01	947
2	2.570	2633549	1.23	435477
3	3.507	735108	0.34	33546
4	5.043	9805	0.00	651
5	6.822	78175	0.04	3779
6	7.045	83478	0.04	5089
7	7.203	66115	0.03	5355
8	7.820	581086	0.27	16595
9	8.749	775713	0.36	25475
10	9.447	325440	0.15	11555
11	10.387	882547	0.41	20400
12	11.017	953840	0.45	28086
13	11.853	2915239	1.36	86837
14	12.467	565867	0.26	27905
15	13.088	924183	0.43	31678
16	13.160	468207	0.22	31979
17	13.766	1224906	0.57	43299
18	14.633	3503010	1.64	84712
19	15.471	1966123	0.92	60085
20	15.728	1016548	0.48	66817
21	16.317	3374916	1.58	110591
22	16.627	663866	0.31	60558
23	17.526	4303710	2.01	88642
24	17.688	741644	0.35	86630
25	18.026	1554964	0.73	90925
26	18.356	1800352	0.84	101584
27	18.852	3360093	1.57	117653
28	19.607	6271811	2.93	186866
29	19.840	4324472	2.02	201700
30	20.208	6132533	2.87	247918
31	20.820	2351992	1.10	151443
32	21.075	3520296	1.65	173642
33	23.221	22630392	10.58	235431
34	23.623	17865849	8.35	1114913
35	24.057	4708441	2.20	290733
36	24.359	4086311	1.91	276895
37	24.661	35429569	16.57	1765464
38	26.639	30653977	14.33	480095
39	26.977	5710437	2.67	393204
40	27.595	12852254	6.01	398867
41	27.911	21792801	10.19	563202

Table 7: HPLC fingerprint of extract AV016BaSu(65)04(100).

	Retention Time	Area	% Area	Height
1	1.241	10089	0.00	743
2	1.737	139563	0.04	29368
3	1.897	10508564	3.02	2120419
4	2.474	168462	0.05	20793
5	3.560	244245	0.07	13079
6	5.156	118208	0.03	5005
7	6.455	515048	0.15	12630
8	7.011	397795	0.11	16720
9	7.792	1203998	0.35	35331
10	8.408	1782066	0.51	81114
11	9.287	3153097	0.90	85209
12	10.010	3363741	0.97	117298
13	10.521	3211695	0.92	129563
14	10.919	3272829	0.94	151739
15	11.201	2910895	0.84	187760
16	11.469	2011099	0.58	187543
17	12.570	22668982	6.51	608697
18	13.072	22315645	6.40	1372355
19	13.943	15908037	4.56	466306
20	14.267	13002097	3.73	755966
21	14.499	14043370	4.03	677681
22	15.168	18500419	5.31	803270
23	15.443	8340109	2.39	561189
24	15.645	7732494	2.22	554586
25	15.999	15671975	4.50	569336
26	16.333	14724201	4.23	556235
27	16.825	11690960	3.35	545534
28	17.178	11285355	3.24	522522
29	18.532	79310513	22.76	612406
30	19.864	57401105	16.47	441406
31	25.389	2531990	0.73	47750
32	29.165	4724	0.00	733
33	29.850	337961	0.10	60806

Table 8: HPLC fingerprint of extract AV016BaSu(65)06(100).

	Retention Time	Area	% Area	Height
1	1.726	325346	0.17	47632
2	1.893	23371224	12.48	3640871
3	2.482	714853	0.38	60900
4	3.177	1875237	1.00	99118
5	3.690	485133	0.26	25416
6	4.103	679406	0.36	27133
7	4.359	352324	0.19	24825
8	4.857	272252	0.15	14769
9	5.596	1486044	0.79	49063
10	6.072	4857288	2.59	165611
11	7.074	715830	0.38	46771
12	7.247	928001	0.50	52209
13	7.925	1065970	0.57	45480
14	8.330	2132695	1.14	79394
15	9.096	2308356	1.23	80800
16	9.743	2396105	1.28	79390
17	11.179	12808928	6.84	205854
18	12.413	25125074	13.42	602504
19	13.130	1899754	1.01	160549
20	14.031	28439970	15.19	935325
21	14.646	9859006	5.27	326112
22	15.208	3351792	1.79	211034
23	15.744	11230538	6.00	540161
24	16.030	4547672	2.43	212887
25	16.651	5194350	2.77	232212
26	16.896	3162049	1.69	216298
27	17.244	5839204	3.12	208158
28	17.782	12488631	6.67	281615
29	18.729	4904610	2.62	146569
30	19.386	2196346	1.17	115299
31	19.673	8273125	4.42	99688
32	21.529	667601	0.36	50722
33	21.763	1946885	1.04	46660
34	22.802	1281629	0.68	26705
35	26.305	16760	0.01	1806
36	27.757	52103	0.03	5397

Table 9: HPLC fingerprint of extract AV016BaSu(105)08(100).

	Retention Time	Area	% Area	Height
1	2.531	251954	2.83	45170
2	3.153	91508	1.03	7618
3	3.590	53827	0.60	2846
4	5.809	95383	1.07	5176
5	6.237	84290	0.95	3408
6	7.377	237161	2.66	7025
7	7.781	208394	2.34	12462
8	7.968	105188	1.18	10471
9	8.523	386758	4.34	15438
10	8.922	255053	2.86	13182
11	9.164	103465	1.16	11779
12	9.356	146409	1.64	11519
13	9.690	263555	2.96	11664
14	10.035	197458	2.22	12951
15	10.220	188840	2.12	14479
16	10.471	188182	2.11	14258
17	10.814	371997	4.18	13832
18	11.142	112966	1.27	12605
19	11.344	318863	3.58	13928
20	11.735	280148	3.15	11849
21	12.451	609274	6.84	21533
22	13.107	585271	6.57	38319
23	13.648	210651	2.37	8967
24	14.205	227248	2.55	13908
25	14.513	2594830	29.14	208042
26	15.951	40742	0.46	3155
27	16.683	52708	0.59	5023
28	17.691	70180	0.79	6181
29	24.062	198328	2.23	4386
30	24.791	13467	0.15	1398
31	24.993	12465	0.14	996
32	25.557	2542	0.03	494
33	26.037	62889	0.71	3648
34	26.578	50279	0.56	5059
35	28.119	5587	0.06	665
36	28.951	42494	0.48	3689
37	29.707	184276	2.07	17288

Table10: HPLC fingerprint of extract AV016Fr(105)08(100).

	Retention Time	Area	% Area	Height
1	2.238	10762330	4.73	1150702
2	2.553	5628824	2.47	655829
3	2.914	2203056	0.97	125091
4	3.208	3512641	1.54	318280
5	3.357	1070868	0.47	212573
6	3.602	1899749	0.84	121669
7	4.052	3407226	1.50	136020
8	4.503	2919013	1.28	94633
9	5.093	2190949	0.96	89619
10	5.889	12371237	5.44	338787
11	6.223	10361831	4.55	315486
12	7.163	4782640	2.10	237340
13	7.700	40604091	17.85	1251589
14	9.163	11851832	5.21	268920
15	9.857	3502688	1.54	228636
16	10.125	4862586	2.14	227948
17	10.448	2710010	1.19	231182
18	10.625	2889073	1.27	233695
19	10.818	1656694	0.73	207765
20	11.218	23908811	10.51	1250520
21	12.067	40804495	17.94	1657258
22	13.556	507546	0.22	127495
23	13.790	6006453	2.64	134107
24	14.940	8534623	3.75	102587
25	17.771	960263	0.42	27876
26	18.313	820905	0.36	25276
27	20.696	13722	0.01	1129
28	23.988	77588	0.03	2021
29	24.355	9612	0.00	1658
30	25.622	204766	0.09	9215
31	25.787	301167	0.13	11470
32	26.472	205806	0.09	9613
33	27.231	486773	0.21	12154
34	28.270	5013669	2.20	94934
35	29.001	2956931	1.30	213028
36	29.400	7494149	3.29	238402

Table 11: MS Fingerprint of extract AV016BaDi(28)04(20)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.1775	1.06E+08	12.3131	1.97E+07	17.7412	0.1874	Valley
2.3167	3.07E+08	35.522	3.45E+07	31.1894	0.2408	Valley
2.8588	2.07E+07	2.398	2.52E+06	2.2768	0.2408	Base to Base
3.2879	1.84E+07	2.1314	2.99E+06	2.7	0.1873	Base to Base
3.7661	1.19E+07	1.3822	1.74E+06	1.5668	0.2408	Base to Base
5.0086	9.58E+06	1.1091	1.08E+06	0.9759	0.2941	Base to Base
6.1538	8.09E+06	0.9365	1.03E+06	0.9294	0.2141	Base to Base
6.8962	6.00E+06	0.6949	1.39E+06	1.2572	0.1605	Base to Base
7.7876	8.31E+06	0.9627	1.05E+06	0.9452	0.2676	Valley
7.9719	1.18E+07	1.3624	1.89E+06	1.7057	0.214	Valley
10.735	8.06E+06	0.9334	1.45E+06	1.3135	0.2408	Base to Base
12.0425	2.48E+06	0.2866	6.74E+05	0.6081	0.107	Base to Base
12.3092	1.54E+06	0.1783	8.28E+05	0.7472	0.0803	Base to Base
12.7311	4.83E+06	0.5597	9.68E+05	0.8739	0.1605	Base to Base
14.3893	3.76E+06	0.4351	9.54E+05	0.8611	0.1605	Base to Base
15.0886	3.66E+06	0.4237	9.12E+05	0.8235	0.1338	Base to Base
17.5521	1.82E+07	2.1125	2.34E+06	2.1145	0.3211	Base to Base
17.9476	1.90E+07	2.1971	1.86E+06	1.6753	0.2408	Base to Base
19.5586	1.44E+07	1.6648	1.53E+06	1.3819	0.3211	Base to Base
20.3886	4.78E+06	0.553	1.22E+06	1.1028	0.1605	Base to Base
21.5431	1.49E+07	1.7195	1.31E+06	1.1854	0.3746	Base to Base
23.6233	8.28E+06	0.9586	1.77E+06	1.5935	0.1873	Base to Base
24.5583	8.58E+06	0.9939	1.18E+06	1.0651	0.2676	Base to Base
25.1938	9.77E+06	1.131	1.24E+06	1.117	0.3211	Base to Base
25.6005	5.11E+07	5.9143	5.26E+06	4.7467	0.3478	Base to Base
25.9137	1.34E+07	1.5528	1.76E+06	1.5921	0.2141	Base to Base
26.7469	1.82E+07	2.1106	2.05E+06	1.8533	0.2943	Base to Base
27.3985	1.09E+07	1.2594	1.39E+06	1.2536	0.2675	Base to Base
27.6349	4.60E+06	0.5329	1.06E+06	0.9601	0.1338	Base to Base
28.7272	1.89E+07	2.1893	1.77E+06	1.5968	0.3478	Base to Base
28.8934	1.03E+07	1.1905	2.09E+06	1.8883	0.1338	Base to Base
29.1613	3.01E+06	0.3489	9.23E+05	0.8329	0.1338	Base to Base
29.9922	8.20E+07	9.495	5.03E+06	4.5397	0.6957	Base to Base
34.628	1.16E+07	1.3485	1.01E+06	0.9137	0.3746	Base to Base
36.8649	2.86E+06	0.3315	8.91E+05	0.804	0.107	Base to Base
37.1874	1.77E+06	0.2051	6.81E+05	0.6146	0.0802	Base to Base
37.7494	4.85E+06	0.5616	7.24E+05	0.6535	0.1873	Base to Base

Table 12: MS Fingerprint of extract AV016BaDi(28)04(20)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.1961	2.85E+08	24.4168	3.04E+07	25.3851	0.2676	Valley
2.4834	4.77E+08	40.8241	2.58E+07	21.5134	0.5084	Valley
3.4214	7.47E+06	0.6387	1.44E+06	1.2067	0.1873	Base to Base
4.4188	3.03E+06	0.2592	1.25E+06	1.0442	0.107	Base to Base
7.9809	8.39E+06	0.7181	1.33E+06	1.1079	0.1873	Base to Base
9.6559	5.75E+06	0.4914	1.14E+06	0.9551	0.1873	Base to Base
11.4246	2.60E+06	0.2223	8.53E+05	0.7128	0.1338	Base to Base
12.387	6.72E+06	0.575	1.29E+06	1.0785	0.1873	Base to Base
12.7886	3.28E+07	2.8027	4.15E+06	3.4643	0.2408	Base to Base
14.0996	3.48E+06	0.2974	1.12E+06	0.9338	0.107	Base to Base
14.8736	3.87E+06	0.3307	5.13E+05	0.4282	0.1605	Base to Base
15.1974	2.00E+06	0.1714	1.08E+06	0.9018	0.0803	Base to Base
16.2118	1.20E+07	1.0275	1.28E+06	1.0691	0.2676	Base to Base
17.1494	6.42E+06	0.5495	1.26E+06	1.0499	0.214	Base to Base
17.3337	1.35E+07	1.157	2.57E+06	2.1439	0.1873	Base to Base
17.6906	3.73E+07	3.1891	2.51E+06	2.0939	0.4013	Valley
17.8922	9.86E+06	0.8435	1.99E+06	1.6607	0.1338	Valley
18.3572	4.74E+06	0.4051	2.29E+06	1.9131	0.0803	Base to Base
18.7319	9.90E+06	0.8468	1.37E+06	1.144	0.2408	Base to Base
19.6901	6.66E+06	0.5693	1.68E+06	1.4049	0.1338	Base to Base
20.1151	1.26E+07	1.082	1.77E+06	1.4749	0.2408	Base to Base
21.084	5.17E+06	0.4419	1.45E+06	1.2082	0.1338	Base to Base
21.4301	6.15E+06	0.5263	2.11E+06	1.7665	0.0803	Valley
21.5887	1.05E+07	0.8972	2.53E+06	2.1134	0.1605	Valley
23.7467	8.39E+06	0.7173	1.24E+06	1.0376	0.2675	Base to Base
23.9916	4.44E+06	0.3795	8.19E+05	0.6845	0.1338	Base to Base
24.7774	6.82E+06	0.5835	1.14E+06	0.9516	0.2943	Base to Base
25.5831	2.43E+07	2.0804	2.38E+06	1.9909	0.3211	Base to Base
25.9059	1.37E+07	1.1725	2.04E+06	1.7081	0.2675	Base to Base
26.2502	1.00E+07	0.8555	1.08E+06	0.9016	0.2408	Base to Base
26.7168	1.72E+07	1.471	2.26E+06	1.8859	0.2408	Valley
26.9142	2.14E+07	1.8288	2.20E+06	1.8395	0.2676	Valley
28.3515	3.10E+07	2.6494	3.47E+06	2.8963	0.4281	Valley
28.6747	2.53E+07	2.1615	3.53E+06	2.9518	0.3478	Valley
28.9455	4.47E+06	0.3823	1.21E+06	1.0119	0.1338	Base to Base
29.6212	7.70E+06	0.6586	7.87E+05	0.6574	0.2408	Base to Base
30.0197	1.10E+07	0.939	2.58E+06	2.1565	0.1873	Base to Base
30.4766	9.79E+06	0.8377	1.86E+06	1.552	0.1605	Base to Base

Table13: MS Fingerprint of extract AV016BaDi(65)04(100)

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.6819	5.13E+07	3.3673	1.07E+07	7.702	0.1873	Base to Base
2.1223	4.05E+07	2.6634	4.33E+06	3.1211	0.2408	Base to Base
2.4137	3.19E+08	20.9505	4.49E+07	32.3621	0.3479	Base to Base
35.37	7.19E+07	4.7236	3.26E+06	2.3544	0.6422	Base to Base
43.1584	2.30E+08	15.0758	1.02E+07	7.3293	0.7759	Base to Base
46.4236	1.38E+07	0.9069	1.66E+06	1.1971	0.3746	Base to Base
48.1523	2.43E+07	1.5953	2.55E+06	1.8369	0.3211	Base to Base
48.5731	7.28E+07	4.7831	6.02E+06	4.3446	0.3746	Base to Base
49.1844	4.59E+07	3.0145	4.22E+06	3.0466	0.4014	Base to Base
49.7221	1.53E+08	10.0401	1.05E+07	7.5644	0.6421	Base to Base
50.41	1.98E+08	13.0047	1.31E+07	9.4266	0.6422	Base to Base
53.0591	1.83E+07	1.2049	2.30E+06	1.6573	0.3478	Base to Base
53.7262	1.26E+08	8.2723	8.93E+06	6.4387	0.5619	Base to Base
55.4126	1.19E+08	7.8049	1.12E+07	8.068	0.4013	Base to Base
55.8032	3.95E+07	2.5926	4.92E+06	3.5508	0.3478	Base to Base

Table 14: MS Fingerprint of extract AV016BaDi(65)04(100).

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.5858	4.95E+08	33.2934	5.09E+07	33.5845	0.3211	Valley
1.6837	4.68E+08	31.4658	4.70E+07	30.9815	0.2675	Valley
2.281	2.71E+08	18.2632	2.23E+07	14.6901	0.3479	Valley
2.3736	1.40E+08	9.4462	2.07E+07	13.6345	0.214	Valley
42.7019	3.33E+06	0.2239	1.08E+06	0.7156	0.2141	Base to Base
51.6555	9.78E+06	0.6583	1.30E+06	0.8605	0.2408	Base to Base
54.1648	7.99E+07	5.3782	4.28E+06	2.8245	0.7224	Base to Base
55.1059	3.72E+06	0.2501	1.06E+06	0.697	0.107	Base to Base
57.6108	6.57E+06	0.4422	1.30E+06	0.855	0.214	Base to Base
58.0334	6.71E+06	0.4517	9.03E+05	0.5959	0.2141	Base to Base
58.9956	1.89E+06	0.127	8.50E+05	0.5609	0.0802	Base to Base

Table 15: MS Fingerprint of extract AV016BaSu(65)09(100).

Peak List

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.6837	2.30E+07	1.9654	2.66E+06	2.8378	0.3211	Base to Base
1.7359	3.23E+08	27.6122	2.10E+07	22.4515	0.6155	Base to Base
2.1817	6.42E+08	54.9509	5.24E+07	55.9679	0.3212	Base to Base
46.2873	2.68E+06	0.2294	1.06E+06	1.1283	0.0802	Base to Base
51.3977	1.34E+07	1.1443	1.86E+06	1.987	0.2408	Base to Base
51.7551	1.14E+07	0.9773	2.30E+06	2.4614	0.1605	Base to Base
53.5882	4.82E+07	4.1246	4.09E+06	4.3665	0.4281	Base to Base
56.8025	3.06E+07	2.6205	2.29E+06	2.4491	0.2943	Base to Base
57.7937	7.45E+07	6.3753	5.94E+06	6.3504	0.4549	Base to Base

Table 16: MS Fingerprint of extract AV016BaSu(65)01(100).

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.9306	6.79E+06	0.9084	1.15E+06	1.8547	0.1873	Base to Base
1.6126	3.36E+07	4.493	1.23E+06	1.9756	0.2943	Base to Base
2.2467	5.48E+08	73.275	2.92E+07	46.9161	0.5887	Base to Base
2.6959	5.61E+06	0.7509	2.37E+06	3.8115	0.0803	Base to Base
8.7292	4.57E+06	0.6115	7.87E+05	1.2645	0.1605	Base to Base
22.1814	1.19E+07	1.5974	9.94E+05	1.5977	0.3478	Base to Base
22.538	5.22E+06	0.6979	8.29E+05	1.3331	0.1873	Base to Base
24.7315	5.89E+06	0.7887	1.07E+06	1.7263	0.1605	Base to Base
26.7098	3.29E+06	0.4408	1.01E+06	1.6234	0.107	Base to Base
28.9401	1.24E+07	1.6572	1.40E+06	2.2533	0.2676	Base to Base
29.316	3.87E+06	0.5185	1.47E+06	2.3673	0.0803	Base to Base
33.8775	4.43E+06	0.5927	1.17E+06	1.883	0.1605	Base to Base
35.7436	4.34E+06	0.5805	8.92E+05	1.4329	0.1873	Base to Base
37.7239	6.26E+06	0.8374	1.17E+06	1.8777	0.214	Base to Base
37.8343	1.99E+06	0.2659	7.72E+05	1.2402	0.107	Base to Base
39.1183	5.39E+06	0.7212	9.96E+05	1.6006	0.1606	Base to Base
44.5179	7.33E+06	0.9815	9.63E+05	1.5473	0.2676	Base to Base
49.8635	3.66E+06	0.4897	8.38E+05	1.347	0.1338	Base to Base
51.9325	3.25E+06	0.4342	1.23E+06	1.9711	0.0803	Base to Base
52.2278	3.39E+06	0.4532	1.25E+06	2.0023	0.1071	Base to Base
52.5806	1.33E+07	1.7735	1.73E+06	2.7832	0.2676	Valley
52.735	1.12E+07	1.5033	1.69E+06	2.7234	0.1873	Valley
53.8915	1.27E+07	1.706	2.22E+06	3.5732	0.2141	Base to Base
54.4726	7.86E+06	1.0524	1.51E+06	2.4278	0.1338	Base to Base
57.8304	1.22E+07	1.6307	2.45E+06	3.9445	0.1873	Base to Base
59.5907	5.79E+06	0.7754	1.11E+06	1.7919	0.1338	Base to Base
59.9224	3.46E+06	0.463	7.03E+05	1.1303	0.1338	Base to Base

Table 17: MS Fingerprint of extract AV016BaSu(65)01(100).

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.9354	4.30E+06	1.2293	1.22E+06	2.4333	0.214	Base to Base
2.2806	1.29E+08	36.7178	1.08E+07	21.6409	0.4549	Base to Base
2.6726	4.11E+07	11.7354	1.62E+07	32.297	0.0803	Base to Base
2.9712	3.79E+07	10.8273	7.74E+06	15.4562	0.1873	Base to Base
51.3175	1.61E+06	0.4586	6.88E+05	1.3736	0.0803	Base to Base
53.8048	2.24E+07	6.3996	1.62E+06	3.2379	0.3211	Base to Base
54.3764	3.18E+07	9.0759	2.29E+06	4.5779	0.5351	Base to Base
56.2363	1.47E+07	4.2035	1.55E+06	3.1017	0.3478	Base to Base
56.9396	2.67E+06	0.7637	1.65E+06	3.2962	0.0535	Base to Base
57.4693	1.54E+07	4.4064	2.12E+06	4.2359	0.2676	Valley
57.8919	4.50E+07	12.8633	3.30E+06	6.595	0.5886	Valley
58.5708	4.62E+06	1.3192	8.79E+05	1.7544	0.2141	Base to Base

Table 18: MS Fingerprint of extract AV016BaSu(65)01(100)ng.

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.2815	2.64E+07	2.56	1.89E+06	2.0165	0.4014	Base to Base
0.6177	2.15E+07	2.0898	4.89E+06	5.2322	0.214	Base to Base
1.4595	6.17E+07	5.9816	5.53E+06	5.911	0.3478	Valley
1.6305	2.63E+07	2.5549	3.33E+06	3.5575	0.1873	Valley
2.2215	5.21E+08	50.5534	3.02E+07	32.3009	0.5352	Base to Base
4.4158	5.86E+06	0.5685	1.43E+06	1.5317	0.1605	Base to Base
10.5664	6.14E+06	0.5957	7.92E+05	0.8469	0.2408	Base to Base
23.1719	7.20E+06	0.6985	1.10E+06	1.1733	0.2141	Base to Base
26.6471	2.14E+06	0.2071	1.07E+06	1.1409	0.0803	Base to Base
28.4723	8.22E+06	0.7973	9.73E+05	1.04	0.2676	Base to Base
29.1292	3.99E+06	0.387	7.08E+05	0.7574	0.1873	Base to Base
31.5736	8.16E+06	0.7917	8.24E+05	0.8813	0.2408	Base to Base
31.6945	2.56E+06	0.2481	7.61E+05	0.8141	0.107	Base to Base
33.9826	1.00E+07	0.9715	1.28E+06	1.3722	0.2943	Base to Base
36.5048	4.65E+06	0.4512	8.70E+05	0.93	0.1338	Base to Base
37.6212	3.23E+06	0.3131	1.19E+06	1.2726	0.107	Base to Base
39.8509	3.18E+06	0.3083	7.69E+05	0.822	0.1338	Base to Base
42.0646	4.16E+06	0.4038	9.70E+05	1.0375	0.1605	Base to Base
42.4115	3.05E+06	0.2954	6.68E+05	0.7142	0.1338	Base to Base
42.5886	5.36E+06	0.5203	1.14E+06	1.2137	0.1338	Base to Base
43.7214	1.66E+06	0.1606	8.64E+05	0.9238	0.0803	Base to Base
49.6266	3.44E+06	0.3332	6.23E+05	0.6663	0.1605	Base to Base
49.8477	1.80E+06	0.175	9.30E+05	0.9949	0.0803	Base to Base
50.2725	2.67E+06	0.2585	1.22E+06	1.3044	0.0803	Base to Base
50.4625	2.57E+06	0.2489	1.25E+06	1.3321	0.0803	Base to Base
51.6971	9.21E+06	0.8936	1.33E+06	1.4175	0.3211	Base to Base
52.2644	3.77E+06	0.3661	1.08E+06	1.1526	0.1338	Base to Base
52.6743	1.05E+07	1.0181	1.27E+06	1.3617	0.3478	Base to Base
53.3229	1.80E+06	0.175	7.11E+05	0.7607	0.0803	Base to Base
54.0466	5.16E+07	5.0087	5.02E+06	5.3685	0.4281	Base to Base
54.5611	7.42E+06	0.7194	1.35E+06	1.439	0.1873	Base to Base
55.786	3.29E+06	0.3187	1.22E+06	1.304	0.107	Base to Base
56.3205	9.29E+06	0.9008	1.81E+06	1.9397	0.1605	Base to Base
56.982	1.01E+07	0.9748	1.91E+06	2.0474	0.1873	Base to Base
57.5849	6.16E+07	5.9742	5.18E+06	5.5408	0.4549	Valley
57.9722	1.07E+08	10.4	6.43E+06	6.8743	0.6421	Valley
59.2956	8.01E+06	0.7772	9.41E+05	1.0067	0.2676	Base to Base

Table 19: MS Fingerprint of extract AV016BaSu(65)01(100)g.

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
0.2462	4.54E+06	0.4516	1.28E+06	1.5367	0.1605	Base to Base
0.7783	2.91E+07	2.896	2.30E+06	2.7508	0.4281	Base to Base
1.4229	1.44E+08	14.3325	1.54E+07	18.4396	0.2676	Valley
1.6808	2.48E+08	24.6093	1.67E+07	20.0141	0.4281	Valley
2.2103	5.05E+08	50.1865	3.12E+07	37.4091	0.5084	Base to Base
2.6741	2.06E+07	2.0484	6.02E+06	7.2093	0.1338	Base to Base
3.5511	4.01E+06	0.3982	1.49E+06	1.7817	0.107	Base to Base
4.8158	1.41E+07	1.4004	2.22E+06	2.6599	0.2676	Base to Base
27.5026	4.41E+06	0.4385	1.10E+06	1.3192	0.2141	Base to Base
53.9115	1.52E+07	1.5124	1.73E+06	2.0716	0.3479	Base to Base
55.1432	1.04E+07	1.0317	1.45E+06	1.735	0.3478	Base to Base
56.3186	2.19E+06	0.2172	8.58E+05	1.0289	0.0803	Base to Base
57.4751	1.54E+06	0.153	7.41E+05	0.8876	0.0803	Base to Base
57.9589	3.26E+06	0.3241	9.65E+05	1.1566	0.107	Base to Base

Table 20: MS Fingerprint of extract AV016BaSu(65)04(100).

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.3402	1.14E+08	17.1348	1.35E+07	13.7133	0.2676	Valley
1.6073	1.74E+07	2.6038	1.93E+06	1.9612	0.1606	Valley
2.2967	1.87E+08	28.0404	1.01E+07	10.3069	0.4817	Base to Base
4.0358	4.53E+06	0.6786	1.01E+06	1.0332	0.1338	Valley
4.1691	3.10E+06	0.4647	1.31E+06	1.3305	0.0803	Valley
4.385	5.02E+06	0.7515	1.42E+06	1.4467	0.1338	Base to Base
4.9989	5.94E+06	0.8892	1.16E+06	1.1771	0.1605	Base to Base
5.3019	3.46E+06	0.5183	1.06E+06	1.0776	0.1338	Base to Base
5.9113	2.78E+06	0.4167	1.30E+06	1.3198	0.0803	Base to Base
6.1778	4.49E+06	0.6721	7.58E+05	0.7719	0.1873	Base to Base
6.3704	2.71E+06	0.4056	7.84E+05	0.7985	0.107	Base to Base
6.8924	6.19E+06	0.9275	7.75E+05	0.7891	0.214	Base to Base
7.2702	3.77E+06	0.5654	1.17E+06	1.1885	0.107	Base to Base
7.4357	2.04E+06	0.3051	9.28E+05	0.945	0.0803	Base to Base
7.9721	4.93E+06	0.7391	9.22E+05	0.939	0.1873	Base to Base
8.8605	6.09E+06	0.9128	7.53E+05	0.7667	0.2141	Base to Base
9.4252	2.87E+06	0.4292	7.85E+05	0.7996	0.1071	Base to Base
9.789	4.10E+06	0.6138	6.12E+05	0.6234	0.2676	Base to Base
10.7492	1.94E+06	0.2902	7.58E+05	0.7718	0.0803	Base to Base
13.0858	2.60E+06	0.3897	6.98E+05	0.7105	0.1094	Valley
13.1888	3.36E+06	0.5029	7.11E+05	0.7238	0.1582	Valley
15.4704	4.27E+06	0.64	1.29E+06	1.3121	0.1338	Base to Base
16.5549	2.37E+06	0.3545	8.66E+05	0.8822	0.0802	Base to Base
17.1515	4.19E+06	0.6275	1.24E+06	1.2608	0.107	Valley
17.2577	5.17E+06	0.7752	9.38E+05	0.9557	0.1606	Valley
17.4256	5.27E+06	0.7899	8.05E+05	0.8196	0.1605	Base to Base
17.7689	5.45E+06	0.816	1.09E+06	1.1149	0.1338	Base to Base
19.3648	2.95E+06	0.4416	8.37E+05	0.8528	0.1606	Base to Base
19.5311	1.19E+06	0.1786	5.57E+05	0.5667	0.0803	Base to Base
20.302	5.74E+06	0.8605	9.81E+05	0.9987	0.1605	Base to Base
23.1422	4.84E+06	0.7254	8.61E+05	0.8767	0.1605	Base to Base
23.4055	3.51E+06	0.5262	7.76E+05	0.7901	0.1345	Base to Base
25.5012	2.20E+06	0.3299	1.04E+06	1.0621	0.0803	Base to Base
25.9266	2.59E+06	0.3882	9.14E+05	0.931	0.107	Base to Base
26.7339	3.31E+06	0.496	1.09E+06	1.1142	0.107	Base to Base
27.8535	3.91E+06	0.5853	1.02E+06	1.0382	0.1873	Base to Base
28.7851	2.65E+06	0.3968	9.95E+05	1.0129	0.0803	Valley
28.9276	5.31E+06	0.7949	8.69E+05	0.8849	0.1873	Valley
29.0503	4.46E+06	0.6684	1.13E+06	1.1496	0.1605	Valley
29.6159	2.76E+06	0.413	1.10E+06	1.1195	0.107	Base to Base
31.7858	2.66E+06	0.3987	7.40E+05	0.7538	0.1338	Valley
31.9243	2.84E+06	0.4248	5.33E+05	0.543	0.1605	Valley
32.5063	3.51E+06	0.5259	1.20E+06	1.2204	0.107	Base to Base
33.128	3.73E+06	0.558	7.30E+05	0.7436	0.2141	Base to Base
33.3651	1.37E+06	0.2059	5.85E+05	0.596	0.0803	Base to Base
34.7807	3.81E+06	0.5705	9.24E+05	0.9412	0.1338	Base to Base
35.2633	2.05E+06	0.3075	7.05E+05	0.7181	0.107	Base to Base
36.9489	5.65E+06	0.8458	9.78E+05	0.9955	0.1605	Base to Base
38.1394	2.87E+06	0.4303	8.43E+05	0.8586	0.107	Base to Base

38.6896	6.18E+06	0.9252	1.03E+06	1.0486	0.2408	Base to Base
40.5898	2.15E+06	0.3224	5.81E+05	0.5917	0.1338	Base to Base
41.5733	7.00E+06	1.0491	9.43E+05	0.96	0.2408	Base to Base
42.0674	3.46E+06	0.5184	8.98E+05	0.9143	0.1338	Base to Base
42.2718	8.14E+06	1.2191	1.05E+06	1.0705	0.2676	Base to Base
42.4327	2.55E+06	0.3818	6.87E+05	0.6998	0.107	Base to Base
44.1144	7.48E+06	1.1211	1.08E+06	1.1033	0.2943	Base to Base
46.1964	6.08E+06	0.9103	8.94E+05	0.9104	0.1873	Base to Base
46.8725	3.21E+06	0.4811	7.64E+05	0.7785	0.1338	Base to Base
47.3852	1.47E+06	0.2207	7.06E+05	0.7194	0.0803	Base to Base
50.6192	1.54E+06	0.2307	6.74E+05	0.6865	0.0803	Base to Base
50.8303	3.42E+06	0.5122	1.03E+06	1.0527	0.107	Base to Base
51.2141	6.26E+06	0.9376	1.25E+06	1.2757	0.1873	Base to Base
51.531	8.74E+06	1.3088	1.14E+06	1.1594	0.2408	Valley
51.6971	7.62E+06	1.1419	1.49E+06	1.5193	0.1606	Valley
51.9366	3.58E+06	0.5358	1.13E+06	1.146	0.1338	Base to Base
52.5223	4.27E+06	0.6399	1.01E+06	1.0277	0.1338	Base to Base
53.2728	4.39E+06	0.6573	7.78E+05	0.7923	0.1606	Base to Base
53.8949	1.32E+07	1.9795	1.63E+06	1.6568	0.3211	Base to Base
54.4009	4.66E+06	0.6975	1.34E+06	1.3633	0.107	Valley
54.5073	1.82E+07	2.7254	1.63E+06	1.6624	0.2676	Valley
55.9786	4.07E+06	0.6101	9.34E+05	0.9515	0.1338	Base to Base
56.3475	1.33E+07	1.9928	1.13E+06	1.1551	0.3211	Valley
56.5847	4.61E+06	0.6907	8.48E+05	0.864	0.1605	Valley
56.8275	9.26E+06	1.3875	1.22E+06	1.243	0.1873	Valley
57.0145	4.88E+06	0.7309	9.98E+05	1.016	0.1606	Valley
57.1188	2.45E+06	0.3669	7.75E+05	0.7891	0.107	Base to Base
57.7155	6.35E+06	0.952	1.11E+06	1.1327	0.1606	Valley
57.8152	9.52E+06	1.4268	1.38E+06	1.4031	0.2141	Valley

Table 21: MS Fingerprint of extract AV016BaSu(65)06(100).

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.3425	1.34E+08	15.6624	2.35E+07	34.2569	0.2141	Base to Base
2.3068	6.22E+08	72.7416	3.24E+07	47.2835	0.5886	Base to Base
4.4357	5.31E+06	0.6201	1.63E+06	2.3849	0.1605	Base to Base
5.2161	2.96E+06	0.3463	1.29E+06	1.8752	0.107	Base to Base
5.6491	9.55E+06	1.1164	1.58E+06	2.3015	0.1873	Base to Base
6.6417	9.07E+06	1.0594	2.04E+06	2.9753	0.1605	Base to Base
17.6827	1.27E+07	1.4888	1.34E+06	1.954	0.4281	Base to Base
54.1984	5.00E+07	5.8447	3.32E+06	4.8386	0.4548	Base to Base
57.3586	9.59E+06	1.1203	1.46E+06	2.1301	0.1873	Base to Base

Table 22: MS Fingerprint of extract AV016BaSu(105)08(100).

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
2.0569	2.14E+08	27.1727	2.15E+07	36.3993	0.2677	Base to Base
3.0165	5.13E+08	65.1587	2.56E+07	43.3466	0.7224	Base to Base
4.4937	1.06E+07	1.3463	2.07E+06	3.4978	0.1873	Base to Base
4.8116	9.88E+06	1.255	1.36E+06	2.304	0.2141	Base to Base
7.1128	9.55E+06	1.2133	1.79E+06	3.0334	0.1606	Base to Base
20.2582	6.97E+06	0.8856	1.05E+06	1.7785	0.1873	Base to Base
21.0285	2.37E+06	0.3008	8.07E+05	1.3647	0.107	Base to Base
21.9949	5.91E+06	0.751	1.41E+06	2.3921	0.1605	Base to Base
26.1397	4.67E+06	0.5933	1.60E+06	2.7139	0.1338	Base to Base
28.479	1.04E+07	1.3233	1.87E+06	3.1697	0.1873	Base to Base

Table 23: MS Fingerprint of extract AV016FrDi(65)04(100).

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.6028	1.52E+09	29.4877	6.12E+07	29.3519	0.6423	Valley
2.2961	3.50E+09	68.0186	1.30E+08	62.3426	0.99	Valley
4.0708	3.33E+06	0.0647	1.35E+06	0.6474	0.107	Base to Base
4.8702	1.71E+07	0.3327	2.48E+06	1.1882	0.2675	Base to Base
5.4024	9.12E+06	0.1773	2.48E+06	1.1914	0.1338	Base to Base
6.2587	4.84E+06	0.0941	1.53E+06	0.7348	0.107	Base to Base
47.0653	5.85E+07	1.1379	2.31E+06	1.1097	0.6689	Base to Base
48.8324	4.52E+06	0.0879	1.54E+06	0.7369	0.1338	Base to Base
49.5832	5.13E+06	0.0998	1.18E+06	0.5668	0.1338	Base to Base
51.2074	3.34E+06	0.065	1.38E+06	0.6601	0.0802	Base to Base
51.9303	6.03E+06	0.1171	1.52E+06	0.7281	0.1338	Base to Base
57.0683	1.63E+07	0.3173	1.55E+06	0.7421	0.3211	Base to Base

Table 24: MS Fingerprint of extract AV016FrSu(105)08(100).

Peak List:

Time (min)	Area (counts)	% Area	Height (cps)	% Height	Width (min)	Baseline Type
1.9597	2.64E+08	29.489	2.96E+07	45.0514	0.2677	Base to Base
3.1068	6.21E+08	69.4326	3.28E+07	49.9315	0.6689	Base to Base
5.6942	4.34E+06	0.485	1.88E+06	2.8549	0.0802	Base to Base
22.1023	5.31E+06	0.5934	1.42E+06	2.1622	0.1605	Base to Base

Table 25. IC_{50} values of antioxidation potential of *T. arjuna* extracts from different plant parts.

Plant Part	Extract-ID	Extraction Description	IC_{50} (µg/ml)
1. Bark	AV016BaDi(65)04(100)	Direct 100% ethanol	26
2. Bark	AV016BaDi(28)04(20)	Direct 20% ethanol	24
3. Bark	AV016BaSu(65)01(100)g	Successive 100% acetone	26
4. Bark	AV016BaSu(65)01(100)ng	Successive 100% acetone	46
5. Bark	AV016BaSu(65)01(100)	Successive 100% acetone	24
6. Bark	AV016BaSu(65)04(100)	Successive 100% ethanol	37
7. Bark	AV016BaSu(65)06(100)	Successive 100% methanol	34
8. Bark	AV016BaSu(105)08(100)	Successive 100% water	46
9. Bark	AV016BaSu(65)09(100)	Successive 100% ethyl acetate	53
10. Fruit	AV016FrDi(65)04(100)	Direct 100% ethanol	34
11. Fruit	AV016FrDi(105)08(100)	Successive 100% water	39
12.	Ascorbic acid (positive control)		26

Table 26. Anti-microbial activity of *Terminalia arjuna* bark successive extracts:

Sr. No.	Organism	Extracts										Control		
		AV016BaSu(65)09 (100)		AV016BaSu(65)01 (100)		AV016BaSu(65)04 (100)		AV016BaSu(65)07 (100)		AV016BaSu(65)08 (100)		LB	LB+ DMSO (5%)	LB + Cipro-floxacin (2 µg/ml)
		1mg/ml	5 mg/ml	1mg/ml	5 mg/ml	1mg/ml	5mg/ml	1mg/ml	5 mg/ml	1mg/ml	5 mg/ml			
	Gram Negative													
1.	*E. coli*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
2.	*P. aeruginosa*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
3.	*K. pneumoniea*	+++	-	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	-
4.	*B. bronchiseptica*	-	-	+	-	+++	-	+++	-	+++	-	+++	+++	-
	Gram Positive													
5.	*S. aureus*	-	-	-	-	-	-	-	-	-	-	+++	+++	-
6.	*S. fecalis*	-	-	-	-	+++	-	+++	-	+++	-	+++	+++	-
7.	*M. luteus*	+++	-	+++	-	+++	-	+++	-	+++	-	+++	+++	-
8.	*B. subtilis*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
9.	*B. cereus*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
10.	*B. pumilus*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-
11.	*S. epidermidis*	+++	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-

+++; abundant growth, ++; growth;+ less growth; -, no growth

Table 27. Anti-bacterial activity of *Terminalia arjuna* fruit extracts:

Sr. No.	Organism	Extracts				Control		
		AV016FrDi(65)04 (100)		AV016FrSu(65)08 (105)		LB	LB+ DMSO (5%)	LB + Cipro-floxacin (2 µg/ml)
		1mg/ml	5 mg/ml	1mg/ml	5 mg/ml			
	Gram Negative							
1.	*E. coli*	+++	++	+++	+++	+++	+++	-
2.	*P. aeruginosa*	+++	++	+++	+++	+++	+++	-
3.	*K. pneumoniea*	+++	+	+++	+++	+++	+++	-
4.	*B. bronchiseptica*	-	-	+++	-	+++	+++	-
	Gram Positive							
5.	*S. aureus*	+++	++	+++	+++	+++	+++	-
6.	*S. fecalis*	+++	++	+++	+++	+++	+++	-
7.	*M. luteus*	+++	++	+++	+++	+++	+++	-
8.	*B. cereus*	+++	-	+++	+++	+++	+++	-
9.	*B. pumilus*	+++	-	+++	+++	+++	+++	-
10.	*S. epidermidis*	+++	-	+++	+++	+++	+++	-

+++; abundant growth, ++; growth;+ less growth; -, no growth

Claims:

1. A method for treating a disease selected from the group comprising cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract in a mammal, which comprises administering to the said mammal an effective non-toxic amount of at least an extract from *Terminalia arjuna* selected from those as defined in Tables 1 – 24.

2. A method for treating infectious diseases in a mammal, which comprises administering to the said mammal an effective non-toxic amount of at least an extract from *Terminalia arjuna* selected from those as defined in Tables 1 – 24.

3. A method according to claim 1 wherein the disease is selected from the group comprising cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract and the extract is selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), or a combination of two or more thereof.

4. A method according to claim 2 wherein the disease is any infectious disease and the extract is selected from the group consisting of

AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), or a combination of two or more thereof.

5. A method according to any one of claims 1 – 4 wherein the said treatment is a prophylactic treatment.

6. A pharmaceutical formulation for use in the treatment of a disease selected from the group consisting of cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract, comprising at least one extract isolated from *Terminalia arjuna*, and selected from those listed in Tables 1 – 24 in admixture with a pharmaceutically acceptable carrier.

7. A pharmaceutical formulation for use in the treatment of any infectious disease, comprising at least one extract isolated from *Terminalia arjuna*, and selected from those listed in Tables 1 – 24 in admixture with a pharmaceutically acceptable carrier.

8. A formulation according to claim 6 for use in the treatment of a disease selected from the group consisting of cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract, comprising at least one extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g,

AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), or a combination of two or more thereof.

9. A formulation according to claim 7 for use in the treatment of any infectious disease, comprising at least one extract selected from the group consisting of, AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), or a combination of two or more thereof.

10. A formulation according to any one of claims 6-9 for prophylactic use.

11. A method for the preparation of a pharmaceutical formulation comprising bringing into association at least an extract of the invention, and a pharmaceutically acceptable carrier therefore.

12. An extract from *Terminalia arjuna* selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24.

13. A comestible comprising at least an extract from *Terminalia arjuna* selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24.

14. A comestible according to claim 13 comprising at least an extract for use in the prophylaxis of a disease selected from the group comprising cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease,

myocardial infarction, senile dementia, retinal degeneration and senile cataract.

15. A comestible according to claim 13 comprising at least an extract for use in the prophylaxis of any infectious disease.

16. Use of an extract selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24 for the preparation of a medicament for the treatment of disease selected from the group consisting of cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract.

17. Use according to claim 16 of an extract selected from the group consisting of AV016BaDi(65)04(100), AV016BaDi(28)04(20), AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)01(100)g, AV016BaSu(65)01(100)ng, AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), for the preparation of a medicament for the treatment or prophylaxis of disease selected from the group consisting of cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract.

18. Use of an extract selected from the group consisting of the extracts having the HPLC and/or MS characteristics shown in Tables 1 – 24 for the preparation of a medicament for the treatment of any infectious disease.

19. Use according to claim 18 of an extract selected from the group consisting of AV016BaSu(65)09(100), AV016BaSu(65)01(100), AV016BaSu(65)04(100), AV016BaSu(65)06(100), AV016BaSu(105)08(100), AV016FrDi(65)04(100) and AV016FrSu(105)08(100), for the preparation of a medicament for the treatment or prophylaxis of any infectious disease.

Abstract

The invention relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing diseases such as cardiovascular disease, diabetes, degenerative neurological diseases, cancer, age related diseases like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, heart diseases, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract; owing to the extracts antioxidation potential.

The invention also relates to extracts from *Terminalia* plant species that are capable of being used in methods for managing various infectious diseases.

More particularly, the invention relates to certain extracts from *Terminalia arjuna*, their uses as antimicrobial and antioxidants agents for the treatment of certain diseases heart disease, diabetes, degenerative neurological diseases, cancer, age related disease like amyloidosis, acute pancreatitis, arthritis, atherosclerosis, cancer, cardiovascular disease, inflammatory bowel disease, myocardial infarction, senile dementia, retinal degeneration and senile cataract in mammals, particularly humans, processes for obtaining them and delivery formats therefore